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Exposures across childhood and their relationship with weight and metabolic status

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EXPOSURES ACROSS CHILDHOOD AND THEIR RELATIONSHIP WITH WEIGHT AND METABOLIC STATUS

by

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For my grandparents, Marcella Marie Walsh and George Walls Jr., two very special people who were my biggest supporters at the start of this journey. I would not be where I am today without them.

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ABSTRACT

Pediatric obesity has reached epidemic proportions. Reducing obesity among children is expected to lower their likelihood of being obese as adults and, therefore, lower their cardiovascular and metabolic risk profile in adulthood including hypertension, dyslipidemia, type II diabetes, heart disease, and stroke.

Pediatric obesity among ages 2–19 is defined as a body mass index (BMI) greater than or equal to the 95th percentile for age and gender as defined according to the CDC BMI-for-age growth charts. Risk factors for obesity are present as early as birth, suggesting exposures at different stages of the life cycle are important to study. The primary objective of this thesis was to evaluate exposures throughout childhood and evaluate their association with both weight and metabolic status.

Study 1 examined the relationship between physical activity and metabolic syndrome in overweight and obese youth ages 12–19. We found that even modest amounts of moderate to vigorous physical activity were associated with a reduction in risk of metabolic syndrome, with time spent in vigorous physical activity driving the association.

Study 2 explored the relationship between environmental tobacco smoke (ETS) exposure and childhood overweight and obesity in 3–6 year old children. We observed that ETS has a positive association with risk of overweight/obesity, with a dose-response effect observed.

Study 3 examined the relationship between maternal antibiotic use during pregnancy and infant birthweight. We did not observe any association between maternal antibiotic use and birthweight or BW/GA-z (birthweight adjusted for gestational age z-score), but we did observe a reduction in risk of SGA (small for gestational age) for infants exposed to antibiotics during gestation. This association was most evident among third trimester users.

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LIST OF ABBREVIATIONS

AHFS	American Hospital Formulary Service
ATPIII	Adult Treatment Panel III
BDS	Birth Defects Study
BMI	Body mass index
BU	Boston University
BW/GA-z	Birthweight adjusted for gestational age z-score
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
DBP	Diastolic blood pressure
DM	Diabetes mellitus
EGIR	European Group for the study of Insulin Resistance
EMM	Effect measure modification
EMR	Electronic medical records
ETS	Environmental tobacco smoke
FPG	Fasting plasma glucose
g	grams
GI	Gastrointestinal
HBW	High birthweight
HDL-C	High-density lipoprotein cholesterol
HEI	Healthy Eating Index
IDF	International Diabetes Federation

IGT	Impaired glucose tolerance
IR	Insulin resistance
lbs	pounds
LBW	Low birthweight
LDL-C	Low-density lipoprotein cholesterol
LMP	Last menstrual period
LTPA	Leisure time physical activity
MDPH	Massachusetts Department of Public Health
MetS	Metabolic syndrome
METs	Metabolic equivalents
MHO	Metabolically healthy obesity
MPA	Moderate physical activity
MUO	Metabolically unhealthy obesity
MVPA	Moderate and vigorous physical activity
NCEP	National Cholesterol Education Program
NEST	Newborn Epigenetic STudy
NHANES	National Health and Nutrition Examination Survey
NOS	Not otherwise specified
OGTT	Oral glucose tolerance test
OR	Odds ratio
PA	Physical activity
ROC	Receiver operating curve

SGA	Small for gestational age
SBP	Systolic blood pressure
SD	Standard deviation
SES	Socioeconomic status
SE	Standard error
SGA	Small for gestational age
T2D	Type 2 diabetes
TG	Triglycerides
TV	Television
VPA	Vigorous physical activity
WC	Waist circumference
WIC	The Special Supplemental Nutrition Program for Women, Infants, and Children

1. INTRODUCTION

Approximately 17% of all children and adolescents ages 2–19 are obese with prevalence nearly equivalent in boys and girls (16.9% vs. 17.1%).[1,2] From 1999–2014, the obesity rates increased from 14.0% to 16.9% for boys and from 13.8% to 17.1% for girls.[1,2] Reducing obesity among children is expected to lower their likelihood of being obese as adults and, therefore, lower their cardiovascular and metabolic risk profile in adulthood including hypertension, dyslipidemia, type II diabetes, heart disease, and stroke.[3] Risk factors for obesity can be present as early as birth. Low birthweight (LBW) and small for gestational age (SGA) infants have a more rapid postnatal catch up period that has been shown to be associated with overweight in early infancy.[4] LBW has also been linked to overweight, obesity, increased risk of cardiovascular disease, and type II diabetes in adulthood.[5] High birthweight (HBW), defined as birthweight >4,000 grams (or 8.8 pounds), is often the result of the mother gaining more than the recommended amount of weight during pregnancy and has also been shown to be associated with increased risk for childhood obesity by age 4.[6,7]

Pediatric obesity in 12–19 year olds is defined as a body mass index (BMI = weight(kg)/[height(m)2]) greater than or equal to the 95th percentile for age and gender according to the CDC BMI-for-age growth charts.[8] The CDC BMI-for-age growth charts reflect the distribution of BMI in a reference population. The reference population used to calculate the growth curves consisted of data from the National Health Examination Survey (NHES) II (1963–1965) and III (1966–1970) and NHANES I (1971–1974), NHANES II (1976–1980) and NHANES III (1988–1994).[9] Overweight in children is

defined as BMI-for-age between the 85th and 95th percentiles as defined by the distribution in the reference population. These criteria differ for children and adults. For adults, overweight is defined as BMI 25–29.9, and obesity is defined as BMI ≥30.[10] These adult criteria are not appropriate to apply to children and teens because the amount of body fat changes with age and differs between girls and boys.[8]

Many causes of childhood obesity have been explored, including poor diet, sedentary lifestyles, infant feeding practices, maternal smoking during pregnancy, and socioeconomic status. The literature on this topic is emerging and is not without limitations. This thesis explores several understudied exposures across different periods of childhood and their relationship with body size, applying methodologies and analytical strategies to strengthen the evidence base for three specific hypotheses. The first study examines the association between physical activity and metabolic syndrome among overweight and obese adolescents in NHANES 2007–2012. The second study, also using NHANES 2007–2012, assesses exposure to environmental tobacco smoke and its association with pediatric overweight/obesity. The third study, using data from the Birth Defects Study, examines maternal antibiotic use during pregnancy and its association with infant birthweight.

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2. THE ASSOCIATION BETWEEN PHYSICAL ACTIVITY AND METABOLIC SYNDROME IN ADOLESCENTS AGES 12–19 IN NHANES 2007–2012

2.1 INTRODUCTION

Obesity is a major risk factor for cardiovascular disease, which is rooted in some individuals in childhood. Overweight and obese children are more likely than their normal weight peers to be overweight and obese adults. Among 6–8 year olds with a BMI $\geq 95^{th}$ percentile in the Bogalusa Heart Study, 83% of females and 78% of males became obese in adulthood.[1] However, evidence suggests that not all obese individuals share the same risk of metabolic or cardiovascular disease. Individuals categorized as obese based on BMI can remain free of metabolic syndrome (MetS), a clustering of hyperglycemia/insulin resistance, obesity, and dyslipidemia, and thereby carry a lower risk for cardiovascular disease and type II diabetes compared to their peers with MetS.[2] Identifying those at greatest risk for these comorbidities can increase efficiency for intervention and help identify those most in need of and most likely to benefit from intervention. Studying adolescents (ages 12–19) is particularly important, as they are likely to carry the dietary and physical activity habits they develop at this age with them into adulthood.[3]

More research has been done on MetS in adults than in children. Until recently, there were no clearly established guidelines defining MetS in children because puberty has an effect on fat distribution and insulin sensitivity and secretion.[4] In 2007, the International Diabetes Federation (IDF) published an age-specific set of guidelines for diagnosing MetS in children based on the following risk factors: waist circumference,

blood pressure, triglycerides, HDL-cholesterol, and fasting glucose (**see Appendix 1**)[5].

Physical activity is one behavioral factor thought to decrease the risk of MetS. However, a recent study shows fewer than half of adolescents ages 12–19 are engaging in the recommended amount of physical activity (≥1 hour per day) and there is substantial variability in meeting these guidelines by race/ethnicity.[6] In this cross-sectional study analyzing 987 adolescents ages 12–19 in NHANES 2011–2012, only 32% of adolescents were engaging in at least one hour of physical activity on a daily basis. Multivariable logistic regression models adjusted for age, sex, BMI, parental marital status, and household poverty ratio showed that Hispanics were 33% less likely than whites to meet the PA guidelines (OR=0.67; 95% CI: 0.43, 1.02), non-Hispanic Blacks were 15% less likely than whites to meet the PA guidelines (OR=0.85; 95% CI: 0.60, 1.20), and Asians were 42% less likely than whites to meet the PA guidelines (OR=0.58; 95% CI: 0.36, 0.92).[6]

After the publication of IDF criteria for MetS in children and adolescents, a few studies on metabolic syndrome and physical activity in children and adolescents were published. A cross-sectional study of 181 8–17 year olds with a BMI ≥85th percentile enrolled in a pediatric weight management clinic in Canada evaluated predictors of metabolically healthy obesity (MHO), defined as the absence of each of the following risk factors: SBP or DBP ≥ 90th percentile for age, sex, and height; TG ≥ 1.25 mmol/L; HDL-C ≤ 1.02 mmol/L; glucose ≥ 5.6 mmol/L).[7] Moderate-to-vigorous physical activity was defined as average minutes per day (over the course of a 4 to 7 day period, with at least one weekend day) spent performing higher intensity sports and activities.

Participants self-reported sports and activities in an activity log. Minutes per day spent in activities identified by researchers as moderate, hard, or very hard in intensity were summed and used to define minutes per day spent performing moderate-to-vigorous intensity PA.[7,8] For every standard deviation increase in MVPA (47 minutes/day), an 80% (OR=1.80; 95% CI: 1.24, 2.62) increase in odds of MHO was observed. This study was limited by a racially and socioeconomically homogeneous study population comprised of primarily Caucasian youths from middle- to upper-class households, and the inability to evaluate the effects of sexual maturation.

Another study looked at the relationship between sedentary behavior (physical inactivity) and MetS in a group of 6–14 year old children and adolescents.[9] MetS was defined as having at least 3 of the following 5 criteria: WC, TG, BP (SBP or DBP), and blood glucose >90th percentile for age and sex, and HDL-C <10th percentile for age and sex. Authors reported an increased risk of MetS among children and adolescents who were sedentary for 5 or more hours daily outside of school compared to those who were sedentary for <5 hours per day outside of school (OR=2.30; 95% CI: 1.30, 4.30).[9] Physical inactivity, or time spent sedentary, was reported by the parent and defined as time spent outside of school watching television, sitting at the computer, or playing video games. However, this study was conducted in an Italian population and the prevalence of MetS was low (4.9% in boys, 3.4% in girls), limiting generalizability, especially to a racially and ethnically heterogeneous population like the US.

Other observational cohort studies reported similar results. In a cross-sectional study, Heshmat, et al.[10] reported that a high level of physical activity (compared to low)

was associated with increased levels of HDL-C, lower BMI z-scores, lower total cholesterol, and lower blood pressure in a nationally representative sample of 5,625 Iranian children and adolescents ages 10 to 18. A high level of physical activity was defined as reporting engaging in activity for at least 30 minutes in duration that might lead to heavy sweating or large increases in breathing or heart rate for at least 3 days in the past week, whereas low physical activity was defined by fewer than 3 days of the previously mentioned physical activity in the past week.[10] In another cross-sectional study, Cardenas, et al.[11] reported that mild leisure time physical activity (LTPA) compared to intense LTPA was positively associated with a continuous score assessing cardiometabolic risk, with the association being strongest in overweight and obese youths in their study of 1,309 Mexican children ages 5 to 17. LTPA was categorized into tertiles (mild: ≤21.72, moderate: 21.73–51.3, and intense: >51.3 mets/hour/week). Metabolic equivalents were calculated from self-report of days per week and minutes or hours per day engaged in sports activities during leisure time, walking for transportation, and popular games.[11] Lastly, Neto, et al.[12] reported that time spent in MVPA was inversely associated with a continuous risk score for metabolic syndrome in a study of 391 Brazilian youths ages 10 to 18. Physical activity was measured with an accelerometer worn by the subjects for 7 consecutive days on their hip. Subjects with at least 4 full days of wear time (≥600 mins/day) were included in the analysis. The counts obtained in different activities were converted into metabolic equivalents (METs) and activities were categorized as light (1.5≤METs<3), moderate (3≤METs<6) and vigorous (≥6 METs). These authors developed a receiver operating characteristic (ROC) curve suggesting that

adolescents should perform at least 80 minutes per day of MVPA to minimize the risk of MetS.[12]

Few studies have examined the association between physical activity and MetS in an adolescent population, and these studies have been limited by homogeneous study populations. This study set out to examine the association of physical activity and MetS among adolescents ages 12–19 in a racially-diverse, high-risk (BMI $\geq 85^{th}$ percentile for age and gender) population and to evaluate the effects of puberty on this association in females.

2.2 METHODS

2.2.1 Data Source

The National Health and Nutrition Examination Survey (NHANES) data from 2007–2012[13] was used for this analysis. NHANES was designed to assess the health and nutritional status of adults and children in the United States. It is unique in that it combines interviews, physical exams, measured anthropometry, and biomarker data. NHANES began as a series of surveys in the 1960s and in 1999 became an annual survey. NHANES constitutes a nationally representative sample of about 5,000 individuals in each survey year.[14]

2.2.2 Study Population and Design

This cross-sectional NHANES investigation uses data from 3,750 males and non-pregnant females ages 12–19 who were interviewed about physical activity and diet and

had clinical exams including blood draws and measurement of weight, height, and waist circumference. Participants ages 12 and older who were examined in the morning session and who had completed at least a 9 hour fast were asked to participate in an oral glucose tolerance test (OGTT). Restricting the sample to participants who fasted, were examined in the morning session, and who completed an OGTT reduced our sample, however, it was necessary in order to accurately classify those with impaired fasting plasma glucose and diabetes mellitus (rather than relying on self-report of previous DM diagnosis). This subgroup yielded 1,603 participants. Of these, 611 children (38%) were overweight or obese (defined as BMI ≥85th percentile for age and sex according to the 2000 CDC Growth Charts for the United States).[15] Among them, 78 were excluded from the analysis because they had at least one missing value for metabolic syndrome (MetS), MVPA, age, sex, race, current smoking status, 2-day dietary recall, and food insecurity. The remaining 533 adolescents constituted the study population.

2.2.3 Study Variables

Outcome-Metabolic Syndrome (MetS)

The primary outcome of interest was metabolic syndrome (MetS). MetS was defined with the aid of the metabolic syndrome guidelines established by the IDF[5] (**see Appendix 1**). MetS was defined as having at least 3 of the following 5 metabolic risk factors: (1) abdominal obesity based on waist circumference, (2) triglycerides (TG) ≥150 mg/dL or use of cholesterol lowering medication, (3) high density lipoprotein cholesterol (HDL-C) <40 mg/dL for 12-15 year olds; HDL-C <40 mg/dL for males 16+ and HDL-C <50 mg/dL for females 16+ or use of cholesterol lowering medication, (4) systolic blood

pressure (SBP) ≥130 mm/Hg and/or diastolic blood pressure ≥85 mm/Hg or use of blood pressure lowering medication, (5) fasting glucose ≥5.6 mmol/L or diagnosis of type II diabetes or taking insulin or pills to lower blood sugar.[5] Waist circumference cut-offs vary according to age. For 16–18 year olds, adult criteria were used: ≥ 94 cm for males and ≥ 80 cm for females. For 12–15 year olds, the lower value was chosen between the adult criteria and the cut-point for the 90th percentile for age and sex.[5, 16] Blood specimens, OGTT, anthropometry measures, and blood pressure readings were collected in the mobile examination center (MEC) by trained health technicians. Blood pressure measurements were taken after the participant had rested quietly for 5 minutes and their maximum level of inflation (MIL) was determined. Three consecutive readings were taken; a fourth was made if one of the first three were interrupted or incomplete. NHANES protocol, standardization procedures, and additional data collection information are documented in detail elsewhere.[17–22]

In some cases, participants had missing values for one or two of the five risk factors, but were still appropriately classified as having MetS or no MetS based on the other non-missing values. Those 8 study subjects were included.

Exposure- Physical Activity

The primary exposure of interest was minutes per week engaged in recreational moderate-to-vigorous physical activity (MVPA), which is MVPA unrelated to work or transportation. The 2008 Physical Activity Guidelines for Americans Summary states that children and adolescents should engage in at least 60 minutes of physical activity daily.[23]

Additionally, at least three of these days should include exercise of vigorous intensity. NHANES collected data on moderate and vigorous physical activity separately in a questionnaire.[14] Participants were asked, "Do you do any vigorous-intensity sports, fitness, or recreational activities that cause large increases in breathing or heart rate like running or basketball for at least 10 minutes continuously?" As a follow up, participants were asked, "In a typical week, on how many days do you do vigorous-intensity sports, fitness, or recreational activities?" Lastly, participants were asked "How much time do you spend doing vigorous-intensity sports, fitness, or recreational activity on a typical day?" The same line of questioning was asked for moderate activity. Moderate-intensity sports, fitness, or recreational activities were referred to as "activities that cause a small increase in breathing or heart rate such as brisk walking, bicycling, swimming, or golf for at least 10 minutes continuously".[14] Minutes per week engaged in vigorous recreational physical activity was calculated by multiplying the days per week by the minutes per day the participant reported engaging in vigorous recreational physical activity. The same calculation was performed for minutes per week engaged in moderate physical activity. Minutes per week engaged in vigorous recreational physical activity was added together with minutes per week engaged in moderate recreational physical activity to get the minutes per week engaged in MVPA. MVPA was categorized according to quartiles of the data: (1) <30 minutes per week, (2) 30 to 179 minutes per week, (3) 180–479 minutes per week, and (4) ≥480 minutes per week. Minutes per week engaged in moderate (MPA) and vigorous PA (VPA) were also examined separately. Categories were defined based on quartiles; however, the median for MPA was 0, therefore 3 categories were examined:

(1) 0 minutes per week, (2) >0 to 150 minutes per week, and (3) >150 minutes per week. More than a quarter of subjects had 0 minutes of VPA resulting in some unevenly sized exposure categories: (1) 0 minutes per week, (2) >0 to 90 minutes per week, (3) >90 to 315 minutes per week, and (4) >315 minutes per week.

Covariates

Potential confounders included age, sex, race (white/non-white), SES of the parents (measured by the family monthly poverty level index which is a ratio of monthly family income to the Department of Health and Human Services poverty guidelines specific to family size), dietary quality (Healthy Eating Index 2010 (HEI-2010) total score[23]; sugar intake (g); total energy (kcal); fast food (number of meals per week)), food insecurity (family received food stamp benefit in the past 12 months) and current smoking status of the adolescent (Y/N). Additionally, whether or not the female had an early first period (first period prior to 12 years of age: Y/N, females who had not reported a first period were considered as not having an early first period) was considered for female participants. Dietary intake was measured using two 24-hour recalls. The first recall was assessed in the 24 hours prior to the in-person interview in the mobile examination center (MEC) and the second was collected 3–10 days later via telephone.[20] Sugar intake (g) and total energy (kcal) were calculated by averaging the total sugar and total energy from each of the two 24-hour recall periods. Nutrient estimates were obtained using the USDA's Food and Nutrient Database for Dietary Studies (FNDDS).[25] The Healthy Eating Index (HEI) measures overall diet quality. It consists of 12 components: (1) total fruit, (2) whole fruit, (3) total vegetables, (4) greens and beans, (5)

whole grains, (6) dairy, (7) total protein foods, (8) seafood and plant proteins, (9) fatty acids, (10) refined grains, (11) sodium, and (12) empty calories. The scores of each component are summed together to create a total score (maximum possible value of 100), with higher values indicating an overall better diet quality. Validation of the HEI-2010 found overall adherence to dietary guidelines to be relatively poor in the NHANES population, with an average score of 45.4 in young adults, compared to 56.1 in older adults.[26]

Information on food security came from the Family Questionnaire and was reported by the head of the household (18+ or emancipated minor). Information on demographics, SES, dietary behavior, and health and medical history was reported on the Sample Person Questionnaire, where participants 16 and older were interviewed directly, and information for participants <16 were reported by an adult proxy.[14]

2.2.4 Data Analysis

These data were analyzed using SAS statistical software (version 9.3, SAS Institute). NHANES survey weights were not taken into account given the specific subsample used. Additionally, the aim was to assess association and not prevalence; therefore, results are specific to this sample.[27, 28]

Descriptive analyses were conducted to examine the distribution and frequencies of the exposure and outcome variables as well as the covariates. Bivariate frequency tables between each potential confounder, outcome, and exposure were examined. Means and standard deviations were calculated for continuous variables. The mean of the continuous MVPA variable (measured in minutes/week) was examined by MetS (yes/no).

Logistic regression was used to examine both the crude and adjusted measure of association between MetS and quartiles of MVPA with the lowest level (<30 minutes per week) serving as the reference category. Potential confounders were added to the model one at a time and the relationship with the exposure and outcome was assessed. If the addition of the confounder altered the measure of association by at least 10%, the confounder was left in the model; if not, it was removed. When a final model was selected, the confounders previously excluded were reexamined before deciding on the final model.

Stratified analyses were conducted to examine effect measure modification (EMM) by sex (male vs. female) and food insecurity (received food stamps in last 12 months: Yes/No). Sample size limitations prohibited stratification by current smoking status (Y/N) and race (white vs. nonwhite).

Separate analyses were also conducted to examine the independent association of moderate physical activity with MetS and vigorous physical activity with MetS. In both cases, the lowest level of activity (0 minutes per week for both MPA and VPA) served as the reference category.

2.3 RESULTS

Among the 533 overweight or obese adolescent 12–19 years old who had fasting laboratory data in NHANES 2007–2012 and non-missing values for covariates, 82 (15.4%) met the criteria for metabolic syndrome (MetS). Demographic characteristics are presented by MetS status in **Table 2.1**. Adolescents identified as having MetS engaged in

103 fewer minutes of MVPA per week compared to their peers who did not have MetS. Adolescents with MetS were more likely to be older (16.4 vs/ 15.2), more likely to be male (68.3% vs. 51.7%), to smoke (19.5% vs. 12.0%, respectively), and to belong to a food insecure home (31.7% vs. 27.5%). BMI percentile was 2.9 percentage points higher in the MetS group compared to the no MetS group. Additionally, adolescents with MetS consumed, on average, 300 kcal and 27 g of sugar more per day than adolescents without MetS and the quality of their overall diet was slightly lower.

Table 2.2 shows the prevalence of individual metabolic risk factors by MetS in this study sample. The most prevalent risk factor among those with MetS was abdominal obesity (93.8%), followed by low HDL-C (85.2%), diabetes or elevated fasting plasma glucose (65.9%), high triglycerides (63.0%), and high blood pressure (23.8%). Among those with MetS, 75.6% had three risk factors, 20.7% had 4 risk factors, and 3.7% had all 5 risk factors. Of note, only about one in four overweight youths without MetS had zero risk factors (23.7%). Almost half (44.6%) had one risk factor of concern and another one-third of this subgroup (31.7%) had two risk factors. Adolescents with MetS had, on average, systolic blood pressure 8 mmHg higher, diastolic blood pressure 5 mmHg higher, triglycerides 82 mg/dL higher, HDL-cholesterol 12 mg/dL lower, fasting glucose 8 mg/dL higher, and waist circumference 14 cm greater than their peers who did not have MetS.

Table 2.3 examines the distribution of demographic covariates and the prevalence of individual metabolic risk factors by category of MVPA (<30 minutes/wk, 30–179 minutes/wk, 180–479 minutes/wk, 480+ minutes/wk). The highest MVPA category

(480+ minutes/week) was largely male (67.6%). The distribution of race/ethnicity was similar across activity levels. The lowest activity group (<30 minutes/week) had the highest prevalence of smokers (17.9%), and was on average older than the other activity groups. As minutes per week engaged in MVPA increased, the prevalence of abdominal obesity and low HDL consistently decreased. A U-shaped relationship was observed between diabetes/elevated fasting plasma glucose, high TG, high BP, total number of metabolic risk factors and categories of MVPA. The prevalence of MetS (3+ risk factors) was 24.3% among participants engaging in <30 minutes of MVPA per week, 12.9% among participants engaging in 30–179 minutes per week, 10.3% among participants engaging in 180–479 minutes per week, and 15.1% among those engaging in 480+ minutes per week.

There was little variability in diet quality between adolescents with and without MetS (**Table 2.4**) as measured by the HEI-2010 based on two days of dietary recall data, however, overall diet quality for both groups was poor with adolescents with MetS scoring 44.2 for HEI-2010 total and adolescents without MetS scoring 46.9. Adolescents with MetS ate more refined grains, as indicated by their lower score, compared to adolescents without MetS (HEI-2010 score 4.4 vs. 5.0 out of 10, respectively) and consumed more empty calories (HEI-2010 score 10.5 vs. 11.8 out of 20, respectively).

Higher levels of MVPA were associated with a lower risk of MetS (**Table 2.5**). After adjusting for age, sex, and average daily grams of sugar consumed, the effect was attenuated, but the inverse association remained. In the fully adjusted model, the MVPA OR (95% CI) for 30–179 minutes per week was 0.56 (0.27, 1.15); for 180–479 minutes

per week was 0.41 (0.20, 0.82), and for 480+ minutes per week was 0.51 (0.26, 0.99). White race, current smoking status, food insecurity, and HEI-2010 total score were not associated with MetS and when added to the model, did not appreciably change the effect estimates; therefore, these covariates were not included in the final model.

Models stratified by sex showed a slightly different effect in males as compared to females, with more evidence of a dose-response effect seen in females. However, confidence intervals for females were wider due to the smaller number of females with MetS. In the fully adjusted model for males (adjusted for age and average daily consumption of sugar) (**Table 2.6a**), the MVPA OR (95% CI) for 30–179 minutes per week was 0.50 (0.20, 1.24); for 180–479 minutes per week was 0.30 (0.12, 0.75), and for 480+ minutes per week was 0.54 (0.24, 1.18). In the fully adjusted model for females (adjusted for age, average daily consumption of sugar, and early age at first period (<12 years old)) (**Table 2.6b**), the MVPA OR (95% CI) for 30–179 minutes per week was 0.68 (0.21, 2.20); for 180–479 minutes per week was 0.73 (0.24, 2.21), and for 480+ minutes per week was 0.26 (0.05, 1.39). Early age at first period did not appreciably alter the association between MVPA and MetS in this sample of overweight and obese 12 to 19-year-old females.

Among those with food insecurity (n=150) (**Table 2.7a**), in the model adjusted for age, sex, and average daily sugar consumption, the MVPA OR (95% CI) for 30–179 minutes per week was 0.27 (0.05, 1.49); for 180–479 minutes per week was 0.65 (0.21, 2.04), and for 480+ minutes per week was 0.33 (0.08, 1.33). In the fully adjusted model for participants who were not food insecure (n=383) (**Table 2.7b**), the MVPA OR (95%

CI) for 30–179 minutes per week was 0.73 (0.32, 1.67); for 180–479 minutes per week was 0.31 (0.12, 0.79), and for 480+ minutes per week was 0.61 (0.28, 1.32). There was no evidence of a dose-response relationship for MVPA and MetS in either food insecurity group.

Models exploring moderate and vigorous activity separately (**Tables 2.8a and 2.8b**) showed that MPA did not have an independent association with MetS. In the fully adjusted model, the MPA OR (95% CI) for >0 to 150 minutes per week was 1.30 (0.70, 2.30) and the OR (95% CI) for >150 minutes per week was 0.96 (0.51, 1.79), relative to the reference group of 0 minutes per week. VPA appeared to be driving the observed association between MVPA and MetS. In the fully adjusted model, the VPA OR (95% CI) for >0 to 90 minutes per week was 0.71 (0.27, 1.85); for >90 to 315 minutes per week was 0.58 (0.30, 1.12); and for >315 minutes per week was 0.51 (0.27, 0.98), all relative to the reference group of 0 minutes per week. The model for VPA was the only time we saw a true dose-response effect, with a steady reduction in risk of MetS with increasing time spent engaging in VPA (Cochran-Armitage one-sided test for trend, p=0.02).

2.4 DISCUSSION

In this diverse sample of overweight and obese 12–19 year olds, we observed that even modest amounts of moderate-to-vigorous physical activity have an inverse association with clusters of cardiovascular risk factors known as the metabolic syndrome. This association remained after adjustment for age, sex, and average daily sugar consumption, which were observed to be predictors of MetS. The inverse relationship

was present among subgroups of boys, girls, and those with and without food insecurity, though confidence intervals were wider due to small numbers in the subgroup analyses. We observed that vigorous physical activity was driving this association. We observed an independent, inverse association between VPA and MetS, but did not observe an association for MPA. However, the magnitude of the association was strongest when combining moderate and vigorous physical activity together, suggesting a synergistic effect between the two. Our findings are consistent with other studies that have shown an inverse association between physical activity and metabolic risk factors.[7, 29–32]

One such study that examined the relationship between physical activity and metabolic risk factors in youths ages 8 to 17 aimed to determine independent predictors of metabolically healthy obesity (MHO), defined as the absence of all metabolic risk factors. Conversely, metabolically unhealthy obesity (MUO) was defined as having at least 1 metabolic risk factor.[7] Prince, et al. reported that 21.5% of participants were MHO, defined as the absence of any cardiovascular risk factors; in our study 107 of 533 (20.1%) participants had 0 metabolic risk factors. In their study, MVPA emerged as the strongest independent predictor of MHO, after adjusting for age and sex. Investigators reported that for every standard deviation increase in MVPA (47 min/day), there was an 80% increase in the odds of a participant being in the MHO group.[7] Our study defined MetS as having 3 or more metabolic risk factors and the absence of MetS was defined as having <3 metabolic risk factors; however, with slightly different outcomes, our model adjusted for age and gender only (**Model 2, Table 2.5**) showed an association of similar magnitude. Additionally, our study examined MVPA as minutes per week, where Prince,

et al. examined MVPA with respect to minutes per day. We observed a stronger association between MVPA and MetS in our model adjusted for age and gender; however, once we added a dietary measure (average daily grams of sugar consumed), the association was slightly attenuated. Physical activity models in the study by Prince, et al. did not adjust for dietary factors. Instead, diet was examined separately as a primary predictor in models adjusted for age and gender. Had investigators adjusted their PA models for diet, it is likely they would have noted some attenuation of their observed effect.

In a longitudinal, prospective study of European preadolescents (the IDEFICS cohort), 3,348 children ages 3 to 10.9 who were free of diabetes and insulin resistance (IR) at baseline, were followed for approximately 2 years.[29] A subset of children (n=1,042) wore an accelerometer and had to have at least 3 measurement days with a minimum of 8 hours of wear time per day. Time spent performing MVPA was identified and categorized into quartiles (Q1: ≤27 min/day; Q2: >27 to ≤38.7 min/day; Q3: >38.7 to ≤54.6 min/day; Q4: >54.6 mins/day). The primary outcome was insulin resistance (HOMA-IR), which was taken in a fasting state. Compared to children performing the lowest level of MVPA (≤27 min/day), children engaging in levels of MVPA >38.7 min/day had a reduction in the odds of developing insulin resistance: Q2: (OR=1.1; 95% CI: 0.7, 1.7); Q3: (OR=0.5; 95% CI: 0.3, 0.9); Q4: (OR=0.7; 95% CI: 0.5, 1.1).[27] Models were adjusted for waist circumference z-score, sex, age, parental SES, sedentary time (hours per day of audio-visual media time), and diet (measured by fat consumption propensity score). The prevalence of IR in this population was 17.8%, which is

comparable to our prevalence of MetS (15.4%). In the IDEFICS cohort study, dietary propensity scores were developed based off of the parental food frequency questionnaire. Additionally, food consumed in the school or day care setting was excluded, making misclassification of dietary habits likely.

An intervention study involving 349 obese 9–16 year olds in the Czech Republic reported strong effects of an intensive regimen of physical activity, along with diet, on metabolic risk factors.[30] Over the course of one month, a 6 kg reduction in weight was observed, along with a 2.33 point reduction in BMI (kg/m^2), a 5.6 cm reduction in waist circumference, a 3.1 mm Hg reduction in SBP, a 3.7 mm Hg reduction in DBP, a 0.69 mmol/L reduction in LDL-C, a 0.19 mmol/L reduction in TG, and a 2.0 mIU/L reduction in insulin, among other outcomes.[30] This study population was slightly younger than ours (mean age 13.7) and higher risk (all participants were obese). This intervention study demonstrates the physiologic benefits of physical activity on metabolic risk factors; however, the sizable effects achieved here cannot easily be generalized. This one-month lifestyle intervention program required children to participant in 5 units of aerobic and resistance training per day, with each unit lasting 50 minutes along with calorie restriction (5,000 kcal for children <10 years old and 7,000 kcal/day for children 10+).[30] This level of physical activity far exceeds daily recommendations in the US and is not sustainable. However, despite the lower levels of physical activity observed in our study, we were still able to detect a strong association between physical activity and metabolic risk factors.

Given the relatively small number of studies available examining children and

adolescents, the following two studies in adults were assessed for comparison purposes. A prospective, observational, cohort study (The SUN Project) examined 10,145 Spanish university graduates free from MetS at baseline.[31] Participants were followed for a minimum of 6 years, and the average age of participants was approximately 36 years old. Intensity of leisure time physical activity (LTPA) was categorized into quartiles. Self-reported physical activity was measured in METs and the intensity of LTPA was calculated using the ratio of METs/week to total hours of LTPA per week, resulting in the average METs/hour of LTPA. MetS was defined according to the IDF criteria for adults.[5] This was nearly identical to our definition of MetS, except we followed the IDF guidelines for children, indicating the same HDL-C cut point for all children regardless of sex (<40 mg/dL; rather than <40 mg/dL for men and <50 mg/dL for women) and an examination of waist circumference percentile in addition to set values. 412 new cases of MetS were identified over the follow up period. Vigorous LTPA was associated with a reduction in risk of MetS compared to the reference group, light LTPA (OR=0.63; 95% CI: 0.44, 0.89). The model was adjusted for age, sex, smoking status, baseline BMI, total energy consumption, adherence to Mediterranean diet, following a special diet, snacking, sugar-sweetened beverage consumption, alcohol intake, French fry intake, fast-food consumption, education, computer use, TV watching, house chores, sleep, physical activity at work, prevalence of CVD and cancer, and total energy expenditure in LTPA per week.[30] The effect observed in our study was slightly stronger in magnitude, although confidence intervals were slightly wider, likely due to our smaller sample size.

Another prospective, observational cohort study, The Copenhagen City Heart

Study, of 3,992 men and women ages 21–98 and free of MetS at baseline, observed that 15.4% of adults developed MetS at 10-year follow-up.[32] Participants engaging in moderate/high LTPA had a 29% lower odds of developing MetS compared to their sedentary counterparts (OR=0.71; 95% CI: 0.50, 1.01). The model adjusted for alcohol consumption, smoking, income, duration of schooling, education, cohabitation, and age, but not diet. A sedentary pattern of activity was characterized by fewer than 2 hours per week of light PA (walking, slow biking, or gardening work). "Moderate + high" physical activity was defined as light PA >4 hours per week or 2–4 hours per week of more vigorous PA (sports that cause perspiration or exhaustion), or more than 4 hours per week of moderate PA or regular heavy exercise.[32] MetS was defined using the latest criteria from the American Heart Association[32]; however, biomarker samples were not measured in a fasting state, which could result in bias and misclassification of the MetS outcome. Our study was able to adjust for diet, define MetS risk factors based on a fasting state, and we observed an effect of even larger magnitude in our adolescent sample.

In addition to the collection of serum lipid levels in a fasting state, there were several other strengths of our study. OGTT was used to define elevated fasting plasma glucose and diabetes, rather than strictly relying on self-report of a previous diabetes mellitus diagnosis. The use of an objective diagnostic indicator alleviates concerns about underreporting and misclassification. Our study population was diverse, with 73% of participants who were non-white. Previous studies were limited by homogeneous, predominantly white study populations. Additionally, previous studies have had limited ability to evaluate the effects of sexual maturation on MetS. Although we were unable to

do so in boys, we were able to examine age at menarche in females. In this population, the risk of MetS among female adolescents who had an early menarche (<12 years old) was similar to risk among female adolescents who did not have an early menarche (≥12 years old).

This study was not without limitations. One of the most important limitations of this study, as is the case with all cross-sectional studies, is that exposure and outcome were assessed at the same time; therefore, we are unable to determine temporality of the association. Specifically, we observed a lower prevalence of MetS with increasing levels of physical activity. Because exposure and outcome were assessed at the same time, there is a possibility of reverse causation. The association could be due to the fact that participants were unable to engage in physical activity due to health concerns (diabetes, high blood pressure, obesity, etc.) related to metabolic syndrome. Another possibility is that overweight and obese children may be embarrassed to exercise because of their weight, associated stigma, or fear of being bullied. However, since several prospective, observational cohort studies reported similar results,[29, 31–32] reverse causation is not a likely a likely explanation for our findings. Additionally, we stratified our analyses on extreme obesity (≥97th percentile vs. 85th to <97th percentile) to examine whether or not the ORs had the same inverse pattern in each stratum. Although the effect was stronger in the 85th to <97th percentile strata, the same inverse association pattern was observed in both groups. These results further refute the likelihood of reverse causality.

Another limitation of our study is that there is lack of consensus on the definition of metabolic syndrome (MetS) in adults (**Appendix 2**) and, to date, the IDF is the only

group to attempt to define MetS in children and adolescents. The World Health Organization (WHO) developed adult criteria for metabolic syndrome first in 1998[34], which required the presence of glucose intolerance. The European Group for the Study of Insulin Resistance, or EGIR[35], released criteria a year later. Those criteria were similar, putting the emphasis on insulin resistance and stating that MetS could not be defined in individuals with diabetes. Over the next several years, the National Cholesterol Education Program (NCEP ATPII) released several versions with the most recent not requiring the presence of any single risk factor, but simply three of the five: obesity, hyperglycemia, high TG, low HDL-C, high blood pressure.[36] In 2007, the International Diabetes Federation (IDF) released criteria for MetS in adults and also included criteria for defining the condition in children.[5] The IDF child/adolescent definition requires the presence of abdominal obesity and at least two other risk factors. Across all sets of criteria, there is little variability in the definition of high triglycerides and low HDL-C. However, there is large variability around defining obesity, blood pressure, hyperglycemia, and insulin resistance. The variability is most pronounced with respect to abdominal obesity. The NCEP ATPIII Guidelines define abdominal obesity in adults as a waist circumference >102 cm for men and >88 cm for women.[36] The IDF Guidelines define abdominal obesity in adults as ≥ 94 cm for men and ≥ 80 cm for women.[5] Given that we were using the adult abdominal obesity criteria for adolescents ages 16–19 in our study, we chose a more conservative approach and used the IDF guidelines for abdominal obesity.

In this study, we decided to most closely follow the definition for MetS put forth

by the IDF, since this was the only criterion that included children and adolescents. However, we did not require abdominal obesity as a component, rather we required any three of the five criteria, which included: obesity, elevated fasting plasma glucose, high triglycerides, low HDL-C, and high blood pressure. We found several participants who did not meet the criteria for obesity, but were overweight and had three or four of the other MetS criteria, so we included them in our case definition. It did not seem appropriate to classify subjects who were overweight (but not obese) with three or four of the MetS criteria as metabolically healthy, or free from metabolic syndrome. Therefore, we chose to consider obesity like the other risk factors and, similar to the NCEP ATPIII criteria, required three of the five factors for a diagnosis of MetS.

Physical activity was self-reported, which could lead to misclassification of exposure. It is possible that children with risk factors for MetS are more likely to over-report their PA or that some participants with MetS risk factors are more likely to exercise because they are actively engaged in treatment for weight loss and metabolic risk reduction and, therefore, may be more accurate self-reporters than their less physically active peers; both scenarios would result in differential misclassification of exposure. Differential misclassification of exposure results in bias with an unpredictable direction. Because physical activity was self-reported, it is also possible that there is random measurement error, or information bias, which would result in a non-differential misclassification of exposure, generally biasing the odds ratio toward the null.

Caution should be used when interpreting the results of our stratified models. Only 26 females were classified as having MetS and only 26 of the 159 subjects with

food insecurity were classified as having MetS, leading to less precise estimates.

In summary, in this study sample of overweight and obese 12–19 year olds, we found evidence of an inverse association between physical activity and metabolic syndrome. Specifically, increasing amounts of moderate-to-vigorous physical activity were associated with a lower odds of metabolic syndrome. In this racially diverse population, results were similar for males and females. Future studies should evaluate this association prospectively, and collect more detailed data on puberty and sexual maturation in both males and females to assess the potential influence of these factors on this relationship.

Table 2.1. Distribution of covariates by metabolic syndrome (MetS) for adolescents 12–19 in NHANES 2007–2012

	MetS (n=82)	No MetS (n=451)
Age (years)	16.4 ± 2.2	15.2 ± 2.2
Sex		
Male	56 (68.3%)	233 (51.7%)
Female	26 (31.7%)	218 (48.3%)
Race/Ethnicity		
White	20 (24.4%)	119 (26.4%)
Black	12 (14.6%)	137 (30.4%)
Hispanic	45 (54.9%)	162 (35.9%)
Other	5 (6.1%)	33 (7.3%)
Smoking Status		
Current smoker	16 (19.5%)	54 (12.0%)
Food insecurity[a]	26 (31.7%)	124 (27.5%)
Moderate-vigorous recreational physical activity (minutes per week)	255.5 ± 325.6	358.7 ± 450.3
Moderate-vigorous recreational physical activity		
<30 minutes/week	30 (36.6%)	93 (20.6%)
30–179 minutes/week	15 (18.3%)	101 (22.4%)
180–479 minutes/week	16 (19.5%)	139 (30.8%)
480+ minutes/week	21 (25.6%)	118 (26.2%)
Moderate physical activity	79.5 ± 131.7	132.2 ± 290.7
Vigorous physical activity	176.1 ± 282.7	226.4 ± 326.5
Poverty ratio[b]	1.7 ± 1.3	2.0 ± 1.5
Fast food consumption		
0 times/week	23 (28.1%)	152 (33.7%)
1–3 times/week	47 (57.3%)	236 (52.3%)
>3 times/week	12 (14.6%)	63 (14.0%)
Energy (kcal)[c]	2169.5 ± 919.5	1869.4 ± 795.2
Sugar (g)[3]	138.7 ± 86.4	111.9 ± 56.4
Healthy Eating Index 2010 (HEI-2010) Total Score	44.2 ± 13.2	46.9 ± 12.2
BMI percentile	96.9 ± 3.3	94.0 ±4 .3

Continuous Variables are presented as Mean ± SD and categorical variables are presented as N (%).

[a]Head of household reported receiving food stamp benefit within the past 12 months.

[b]Monthly family income divided by poverty guidelines specific to household size. A value <1 indicates the household is living below the poverty level, a value of 1 indicates the house is living at the poverty level, and a value >1 indicates the household is living above the poverty level. Higher values indicate greater financial security.

[c]Average daily energy and sugar consumption were obtained from a 2-day dietary recall. If a subject was missing one day, a single day of data was used.

Table 2.2. Prevalence of individual metabolic risk factors by MetS in adolescents 12–19 in NHANES 2007–2012

	MetS (n=82) n (%)	No Metabolic Syndrome (n=451) n (%)
Abdominal obesity		
Yes	76 (93.8%)	276 (61.3%)
Waist circumference (cm)	106.9±12.9	92.9±12.7
Low HDL-C		
Yes	69 (85.2%)	87 (19.4%)
HDL-C (mg/dL)	37.2±6.4	49.4±9.6
Diabetes or elevated fasting plasma glucose		
Yes	54 (65.9%)	89 (19.7%)
Fasting glucose (mg/dL)	102.4±13.7	94.7±11.0
High TG		
Yes	51 (63.0%)	23 (5.1%)
Triglycerides (mg/dL)	165.2±81.1	83.0±41.5
High blood pressure		
Yes	19 (23.8%)	12 (2.7%)
SBP (mm Hg)	118.5±12.5	110.3±10.0
DBP (mm Hg)	63.1±12.6	58.0±12.1
Metabolic risk factors*		
0	---	107 (23.7%)
1	---	201 (44.6%)
2	---	143 (31.7%)
3	62 (75.6%)	---
4	17 (20.7%)	---
5	3 (3.7%)	---

*Subjects were classified based on non-missing metabolic risk factors. Subjects having 3 or 4 metabolic risk factors were appropriately classified as MetS, even if they had missing values for 1 or 2 risk factors.

HDL-C=high density lipoprotein cholesterol; TG=triglycerides; SBP= Systolic Blood Pressure; DBP= Diastolic Blood Pressure

Table 2.3. Prevalence of demographics and individual metabolic risk factors by MVPA

	Minutes per week of moderate-to-vigorous physical activity (MVPA)			
	<30 mins (n=123) n (%)	**30–179 mins (n=116) n (%)**	**180–479 mins (n=155) n (%)**	**480+ mins (n=139) n (%)**
DEMOGRAPHICS				
Age (years)	16.3 ± 2.2	14.9 ± 2.3	15.0 ± 2.2	15.5 ± 2.1
Male	50 (40.7%)	56 (48.3%)	89 (57.4%)	94 (67.6%)
Race/Ethnicity				
White	29 (23.6%)	29 (25.0%)	37 (23.9%)	44 (31.7%)
Black	33 (26.8%)	26 (22.4%)	49 (31.6%)	41 (29.5%)
Hispanic	54 (43.9%)	49 (42.2%)	55 (35.5%)	49 (35.3%)
Other	7 (5.7%)	12 (10.3%)	14 (9.0%)	5 (3.6%)
Current smoker	22 (17.9%)	15 (12.9%)	15 (9.7%)	18 (13.0%)
Food insecurity[a]	36 (29.3%)	31 (26.7%)	48 (31.0%)	35 (25.2%)
Poverty ratio[c]	1.7 ± 1.4	1.9 ± 1.5	1.8 ± 1.4	2.3 ± 1.5
Fast food consumption				
0 times/week	46 (37.4%)	40 (34.5%)	48 (31.0%)	41 (29.5%)
1–3 times/week	54 (43.9%)	62 (53.5%)	93 (6.0%)	74 (53.2%)
>3 times/week	23 (18.7%)	14 (12.1%)	14 (9.0%)	24 (17.3%)
Energy[c]	1946 ± 821	1889 ± 825	1824 ± 796	2012 ± 844
Sugar[c]	123 ± 74	106 ± 53	110 ± 55	124 ± 66
HEI-2010 total score	46.2 ± 13.7	46.1 ± 1.9	46.3 ± 12.0	47.3 ± 11.1
METABOLIC RISK FACTORS				
Abdominal obesity				
Yes	102 (82.9%)	83 (72.2%)	89 (57.8%)	78 (56.1%)
Low HDL-C				
Yes	48 (39.3%)	36 (31.0%)	40 (25.8%)	32 (23.4%)
Diabetes or elevated fasting plasma glucose				
Yes	38 (30.9%)	33 (28.5%)	37 (23.9%)	35 (25.2%)
High TG				
Yes	22 (18.0%)	15 (12.9%)	19 (12.3%)	18 (13.1%)
High blood pressure				
Yes	9 (7.3%)	6 (5.3%)	7 (4.5%)	9 (6.5%)
Metabolic risk factors*				
0	12 (9.8%)	17 (14.7 %)	38 (24.5%)	40 (28.8%)
1	39 (31.7%)	47 (40.5%)	59 (38.1%)	56 (40.3%)
2	42 (34.2%)	37 (31.9%)	42 (27.1%)	24 (15.8%)
3 or more	30 (24.3%)	15 (12.9%)	16 (10.3%)	21 (15.1%)

[a]Head of household reported receiving food stamp benefit within the past 12 months.

[b]Monthly family income divided by poverty guidelines specific to household size. A value <1 indicates the household is living below the poverty level, a value of 1 indicates the house is living at the poverty level, and a value >1 indicates the household is living above the poverty level. Higher values indicate greater financial security.
[c]Average daily energy and sugar consumption were obtained from a 2-day dietary recall. If a subject was missing one day, a single day of data was used.
*Subjects were classified based on non-missing metabolic risk factors. Subjects having 3 or 4 metabolic risk factors were appropriately classified as MetS, even if they had missing values for 1 or 2 risk factors.
HDL-C= high density lipoprotein cholesterol; TG=triglycerides

Table 2.4. Component and total 2-day scores for participants with and without MetS, using the Healthy Eating Index 2010 (HEI-2010), adolescents 12–19, NHANES 2007–2012

Component	Max Points	MetS (n=82) Mean (SD)	No MetS (n=451) Mean (SD)
Total vegetables[a]	5	2.5 (1.4)	2.4 (1.4)
Greens and beans[a]	5	1.1 (1.8)	0.9 (1.8)
Total fruit[b]	5	2.0 (1.8)	2.3 (2.0)
Whole fruit[c]	5	1.8 (2.0)	1.9 (2.2)
Whole grains	10	1.8 (2.0)	2.0 (2.6)
Total dairy[d]	10	6.1 (3.3)	6.3 (3.2)
Total protein foods[e]	5	4.3 (1.1)	4.2 (1.2)
Seafood and plant proteins[e,f]	5	1.4 (1.8)	1.4 (1.8)
Fatty acids[g]	10	4.2 (3.2)	4.6 (3.3)
Refined grains	10	4.4 (3.3)	5.0 (3.4)
Sodium	10	4.3 (3.1)	4.1 (3.2)
Empty calories[h]	20	10.5 (6.2)	11.8 (5.4)
HEI-2010 Total Score	100	44.2 (13.2)	46.9 (12.2)

*For subjects with only 1 day of recall, the appropriate HEI-2010 SAS code was used to calculate HEI total score.

[a]Includes any beans and peas not counted as Total Protein Foods.

[b]Includes fruit juice.

[c]Includes all forms except juice.

[d]Includes all milk products such as fluid milk, yogurt, cheese, and fortified soy beverages.

[e]Beans and peas are included here (and not with vegetables) when the Total Protein Foods standard is otherwise not met.

[f]Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods.

[g]Ratio of poly- and monounsaturated fatty acids to saturated fatty acids.

[h]Calories from solid fats, alcohol, and added sugars; threshold for counting alcohol is >13 grams/1000kcal.

Table 2.5 The association between MVPA and MetS in overweight and obese 12–19 year olds in NHANES 2007–2012 (n=533)

Odds Ratio (95% Confidence Interval) for Metabolic Syndrome (MetS)				
Moderate-vigorous recreational physical activity	**n**	**Model 1[a] OR (95% CI)**	**Model 2[b] OR (95% CI)**	**Model 3[c] OR (95% CI)**
<30 mins/week	123	1.00	1.00	1.00
30–179 mins/week	116	0.46 (0.23, 0.91)	0.53 (0.26, 1.07)	0.56 (0.27, 1.15)
180–479 mins/week	155	0.36 (0.18, 0.69)	0.38 (0.19, 0.77)	0.41 (0.20, 0.82)
480+ mins/week	139	0.55 (0.30, 1.03)	0.50 (0.26, 0.97)	0.51 (0.26, 0.99)

[a]Unadjusted
[b]Adjusted for age, sex
[c] Adjusted for age, sex, and average grams sugar consumed

Table 2.6a The association between MVPA and MetS in overweight and obese 12–19 year old males in NHANES 2007–2012 (n=289)

Odds Ratio (95% Confidence Interval) for Metabolic Syndrome (MetS)				
Moderate–vigorous recreational physical activity	**n**	**Model 1[a] OR (95% CI)**	**Model 2[b] OR (95% CI)**	**Model 3[c] OR (95% CI)**
<30 mins/week	50	1.00 (reference)	1.00 (reference)	1.00 (reference)
30–179 mins/week	56	0.42 (0.17, 1.04)	0.48 (0.19, 1.21)	0.50 (0.20, 1.24)
180–479 mins/week	89	0.25 (0.10, 0.59)	0.30 (0.12, 0.73)	0.30 (0.12, 0.75)
480+ mins/week	94	0.49 (0.23, 1.06)	0.53 (0.24, 1.16)	0.54 (0.24, 1.18)
Age			1.18 (1.03, 1.36)	1.18 (1.03, 1.36)
Average daily grams sugar consumed				1.002 (0.997, 1.006)

[a] Unadjusted
[b] Adjusted for age
[c] Adjusted for age and average grams sugar consumed

Table 2.6b The association between MVPA and MetS in overweight and obese 12–19 year old females in NHANES 2007–2012 (n=244)

Odds Ratio (95% Confidence Interval) for Metabolic Syndrome (MetS)					
Moderate–vigorous recreational physical activity	**n**	**Model 1[a] OR (95% CI)**	**Model 2[b] OR (95% CI)**	**Model 3[c] OR (95% CI)**	**Model 4[d] OR (95% CI)**
<30 mins/week	73	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
30–179 mins/week	60	0.42 (0.14, 1.25)	0.61 (0.19, 1.90)	0.59 (0.19, 1.86)	0.68 (0.21, 2.20)
180–479 mins/week	66	0.46 (0.17, 1.30)	0.61 (0.21, 1.76)	0.63 (0.22, 1.83)	0.73 (0.24, 2.21)
480+ mins/week	45	0.22 (0.05, 1.00)	0.30 (0.06, 1.45)	0.32 (0.07, 1.53)	0.26 (0.05, 1.39)
Age			1.26 (1.03, 1.55)	1.25 (1.02, 1.54)	1.21 (0.98, 1.49)
Early menarche (< 12 years old)				0.76 (0.31, 1.84)	0.71 (0.28, 1.76)
Average daily grams sugar consumed					1.01 (1.00, 1.02)

[a] Unadjusted
[b] Adjusted for age
[c] Adjusted for age and early period
[d] Adjusted for age, early period, and average daily grams of sugar consumed

Table 2.7a The association between MVPA and MetS in overweight and obese 12–19 year olds in NHANES 2007–2012: Food Insecure (received food stamps in past 12 months (n=150)

Odds Ratio (95% Confidence Interval) for Metabolic Syndrome (MetS)				
Moderate–vigorous recreational physical activity	**n**	**Model 1[a] OR (95% CI)**	**Model 2[b] OR (95% CI)**	**Model 3[c] OR (95% CI)**
<30 mins/week	36	1.00 (reference)	1.00 (reference)	1.00 (reference)
30–179 mins/week	31	0.14 (0.03, 0.68)	0.20 (0.04, 1.09)	0.27 (0.05, 1.49)
180–479 mins/week	48	0.40 (0.14, 1.12)	0.48 (0.16, 1.44)	0.65 (0.21, 2.04)
480+ mins/week	35	0.26 (0.07, 0.90)	0.31 (0.08, 1.19)	0.33 (0.08, 1.33)
Age			1.23 (0.99, 1.54)	1.20 (0.96, 1.50)
Male			1.33 (0.52, 3.38)	1.11 (0.42, 2.93)
Average daily grams sugar consumed				1.01 (1.00, 1.01)

[a] Unadjusted
[b] Adjusted for age, sex
[c] Adjusted for age, sex, and average grams sugar consumed

Table 2.7b The association between MVPA and MetS in overweight and obese 12–19 year olds in NHANES 2007–2012: Not Food Insecure (did not receive food stamps in past 12 months (n=383)

Odds Ratio (95% Confidence Interval) for Metabolic Syndrome (MetS)				
Moderate–vigorous recreational physical activity	**n**	**Model 1[a] OR (95% CI)**	**Model 2[b] OR (95% CI)**	**Model 3[c] OR (95% CI)**
<30 mins/week	87	1.00 (reference)	1.00 (reference)	1.00 (reference)
30–179 mins/week	85	0.69 (0.32, 1.52)	0.73 (0.32, 1.66)	0.73 (0.32, 1.67)
180–479 mins/week	107	0.31 (0.13, 0.75)	0.31 (0.12, 0.79)	0.31 (0.12, 0.79)
480+ mins/week	104	0.75 (0.36, 1.56)	0.61 (0.28, 1.34)	0.61 (0.28, 1.32)
Age			1.18 (1.03, 1.35)	1.18 (1.03, 1.35)
Male			3.11 (1.56, 6.18)	2.97 (1.48, 5.96)
Average daily grams sugar consumed				1.002 (0.997, 1.007)

[a] Unadjusted
[b] Adjusted for age, sex
[c] Adjusted for age, sex, and average grams sugar consumed

Table 2.8a The association between *Moderate* Physical Activity and MetS in overweight and obese 12–19 year olds in NHANES 2007–2012 (n=533)

Odds Ratio (95% Confidence Interval) for Metabolic Syndrome (MetS)			
Moderate recreational physical activity	**n**	**Model 1[a] OR (95% CI)**	**Model 2[b] OR (95% CI)**
0 mins/week	281	1.00	1.00
>0 to 150 mins/week	131	1.03 (0.58, 1.81)	1.30 (0.70, 2.30)
>150 mins/week	121	0.88 (0.48, 1.61)	0.96 (0.51, 1.79)

[a]Unadjusted
[b]Adjusted for age, sex, and average grams sugar consumed

Table 2.8b The association between *Vigorous* Physical Activity and MetS in overweight and obese 12–19 year olds in NHANES 2007–2012 (n=533)

Odds Ratio (95% Confidence Interval) for Metabolic Syndrome (MetS)			
Vigorous recreational physical activity	**n**	**Model 1[a] OR (95% CI)**	**Model 2[b] OR (95% CI)**
0 mins/week	210	1.00	1.00
>0 to 90 mins/week	52	0.52 (0.21, 1.30)	0.71 (0.27, 1.85)
>90 to 315 mins/week	134	0.54 (0.29, 1.01)	0.58 (0.30, 1.12)
>315 mins/week	137	0.61 (0.33, 1.10)	0.51 (0.27, 0.98)

[a]Unadjusted
[b]Adjusted for age, sex, and average grams sugar consumed

2.5 APPENDIX

Appendix 2.1 Guidelines for Diagnosing MetS in Children[5]

Age, years	Obesity	Triglycerides	HDL-C	Blood Pressure	Fasting Glucose
6 to <10	WC ≥90th percentile	Metabolic syndrome cannot be diagnosed in this age group.			
10 to <16	WC ≥90th percentile	TG ≥150 mg/dL	<40 mg/dL	SBP ≥130 mmHg DBP ≥85 mmHg	≥100 mg/dL or diagnosed T2D (If ≥100 mg/dL [or T2DM] OGTT recommended)
16+ (same as adults)	WC ≥94 cm (males) WC ≥80 cm (females)	TG ≥150 mg/dL or on treatment for lipid abnormalities	<40 mg/dL (males) <50 mg/dL (females) or on treatment for lipid abnormalities	SBP ≥130 mmHg DBP ≥85 mmHg or on treatment for hypertension	≥100 mg/dL or diagnosed T2D

WC=waist circumference
SBP=systolic blood pressure
DBP=diastolic blood pressure
T2D=type II diabetes
OGTT=oral glucose tolerance test

Appendix 2.2 Comparison of Criteria for Diagnosing MetS in Adults[2, 5, 34–37]

	WHO (1998)[34]	EGIR (1999)[35]	NCEP ATP III (2005)[36]	IDF (2007)[5]
Required	Glucose intolerance; IGT; DM	Hyperinsulinemia (fasting plasma insulin ≥75th percentile)	None	Central obesity
Criteria	Glucose intolerance plus ≥2 of the following	Hyperinsulinemia plus ≥2 of the following	≥ 3 of the following 5 criteria	Central obesity plus at least 2 of the following
Obesity	Waist-to-hip ratio: >0.90 M, >0.85 F; BMI>30 kg/m^2	WC ≥94 cm M; ≥90 cm F	WC >102 cm M; >88 cm F	(Required) WC≥94 cm M; WC≥80 cm F
FPG/ Hyperglycemia/ IR	Required	FPG ≥110 mg/dL but nondiabetic	FPG ≥100 mg/dL or Rx	FPG ≥100 mg/dL or Rx
TG	≥150 mg/dL	≥180 mg/dL or Rx	≥150 mg/dL or Rx	≥150 mg/dL or Rx
HDL-C	<35 mg/dL M; <39 mg/dL F	<39 mg/dL or Rx	<40 mg/dL M; <50 mg/dL F; or Rx	<40 mg/dL M; <50 mg/dL F; or Rx
Blood Pressure	≥160/90 mmHg	≥ 140/90 mmHg or Rx	SBP: ≥130 mmHg and/or DBP: ≥85 mmHg	SBP: ≥130 mmHg and/or DBP: ≥85 mmHg
Other	Microalbuminuria (urinary albumin excretion rate ≥20 ug/min or albumin-to-creatinine ratio ≥20 mg/g)	None	None	None

M=males; F=females
IGT=Impaired glucose intolerance; FPG= Fasting plasma glucose
IR=Insulin resistance; TG=Triglycerides; HDL-C= High density lipoprotein cholesterol; DM=Diabetes mellitus; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; WC=waist circumference

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3. THE ASSOCIATION BETWEEN ENVIRONMENTAL TOBACCO SMOKE EXPOSURE AND CHILDHOOD OVERWEIGHT AND OBESITY

3.1 INTRODUCTION

Active smoking has been shown to be associated with lower BMI when comparing active smokers to non-smokers; however, research suggests that although BMI may be lower among smokers, an adverse fat distribution exists compared to non-smokers.[1] A prospective cohort study conducted in 22,059 Greek adults ages 25–84 showed a lower BMI in smokers compared to non-smokers, but within smokers, BMI increased with increasing level of smoking. Additionally, among men, higher waist-to-hip ratios were observed among smokers compared to non-smokers, indicative of abdominal obesity.[1]

Research also shows an increased risk for higher BMI and metabolic syndrome (MetS) associated with passive smoking among adults.[2] In a cross-sectional study of 389 adults from 304 randomly selected Chinese households, exposure to environmental tobacco smoke (ETS) was associated with higher BMI and peripheral and central fat mass accumulation as well as an increased risk of MetS and hypertriglyceridemia.[2] In this study, ETS was assessed via self-reported response to the following question: "Think about the past seven days (one week). On how many of those days were you in a room or vehicle (buses, cars, ships, trains) with someone who was smoking?" Answers were dichotomized ≤ 4 days vs. 5–7 days. The subjective measure of ETS in this study raises concern about misclassification of exposure and potentially biased results.

A similar association between BMI and passive smoking may also exist among

children and adolescents. In another prospective cohort study, researchers showed that children exposed to second hand smoke (SHS) had a greater increase in BMI from age 10 to 18 compared to unexposed children; a dose-response relationship was observed for the number of smokers living in the household.[3] This study, like the previous, was also limited by self-reported SHS exposure. Parents were asked whether or not there was a smoker living in the child's home, if there ever was a smoker living in the home in the past, and the number of smoker(s) currently living in the child's home, if any.

The PIAMA (Prevention and Incidence of Asthma and Mite Allergy) birth cohort study, a prospective study examining 1,687 Dutch children born in 1996 and 1997, assessed predictors of childhood overweight at age 8. Smoking in the household was a strong independent positive predictor: OR=1.73 (95% CI: 1.47, 2.04).[4] Other predictors in the model included father's BMI, mother's BMI, birthweight (kg), female sex, and hospital delivery (vs. at home delivery). The household smoke exposure was assessed via self-report and was dichotomized as smoking in the parental house (Y/N). The investigators did collect information on breastfeeding history and maternal vegetable consumption during pregnancy; however, these covariates were not included in the final model. No other information on dietary habits was collected, and information on physical activity was not collected.

The Quebec Longitudinal Study of Child Development examined household smoke and adiposity measures at age ten.[5] Parents were asked about smoking in the household at four time points throughout the child's first 7 years. A 'No' response at all time points was defined as unexposed, a 'Yes' at one or more of the time points was

defined as transient exposure, and a 'Yes' at all four time points was defined as continuous exposure to ETS throughout childhood. In the fully adjusted multivariable logistic regression model adjusted for maternal smoking during pregnancy, maternal BMI (calculated from maternal report of height and weight at 17-month follow up appointment post-delivery), infant birthweight for gestational age, and maternal immigration status, transient household smoke exposure was associated with a 43% increase in odds of being overweight/obese at age 10 (OR=1.43 [95% CI: 1.12, 1.81]) and continuous household smoke exposure was associated with a 34% increase in odds of being overweight/obese at age 10 (OR=1.34 [95% CI: 0.90, 1.99]) compared to the unexposed group.[5] Data were collected on maternal depressive symptoms (at 5-month follow-up), family income when the child was 5, maternal education, marital status, maternal age at birth of child, alcohol or drug use during pregnancy, child soft drink and snack intake (at 29-month follow-up), and child's television watching habits (at 53-month follow-up). These potential confounders did not considerably alter the measures of effect and, therefore, were not included in the final model. Smoke exposure in this study was measured and classified based on parental self-report. Although one component of physical inactivity was captured in a proxy measure of TV viewing habits, physical activity was not measured and could potentially be a source of unmeasured confounding.

Serum cotinine is a biomarker that provides an objective measure of exposure to environmental tobacco smoke. A major metabolite of nicotine, higher levels of serum cotinine indicate higher exposure to second-hand smoke. One study that used serum cotinine to define ETS was conducted in the NHANES III (survey years 1988–1994)

population where investigators examined the association between smoke exposure and metabolic syndrome (MetS) in 2,006 adolescents ages 12 to 19.[6] Smoke exposure was divided into three categories: non-exposed, exposed to ETS, and active smoking. The non-exposed group was comprised of those with serum cotinine levels below the level of detection (<0.05 ng/mL) and no report of smokers living in the household. The second category was defined according to measurable serum cotinine levels (≤15 ng/mL and no report of smoking in the past 5 days), and the third category was comprised of adolescents who actively smoked (serum cotinine >15 ng/mL or self-report of smoking in the past 5 days).[6] The non-exposed group accounted for 11.8% of the sample population, while the ETS-exposed group accounted for 67% and the active smoking group accounted for 21.2%. Results from a multivariable logistic regression analysis adjusted for sex, age, race/ethnicity, poverty status, region, and parental history of diabetes or heart attack showed a near five-fold increase in the risk of MetS for adolescents with ETS compare to the unexposed group (OR=4.7; 95% CI: 1.7, 12.9) and over a 6-fold increase in risk of MetS for adolescents who were active smokers (OR=6.1; 95% CI: 2.8, 13.4).[6] Individual components of MetS and their association with passive smoke exposure were also examined, with strong positive associations seen for high triglycerides, low HDL, and high waist circumference; a marginal association was also seen for overweight.[6]

The majority of prior studies that examined the effect that ETS exposure has on childhood risk of overweight and obesity largely relied on parental self-report of an exposure that is difficult to quantify. Only one study measured exposure by serum cotinine levels. Further, we are not aware of any studies that have examined this

relationship using serum cotinine to define exposure in a young child. This study examined exposure to ETS and its relationship with overweight and obesity among 3–6 year olds who participated in NHANES 2007–2012 using serum cotinine as the measure of environmental smoke exposure.

3.2 METHODS

3.2.1 Data Source

The National Health and Nutrition Examination Survey (NHANES) data collected from 2007–2012[7] was used for this analysis. NHANES was designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it collects interviews, physical exams, measured anthropometry, and biomarker data. NHANES began in the 1960s and in 1999 became a continuous program.[8–13] NHANES draws a nationally representative sample of about 5,000 individuals each year.[8]

3.2.2 Study Population and Design

In this cross-sectional study combining data from 2007–2012 survey years, 2,563 males and females ages 3–6 were examined and 1,565 had a laboratory value for serum cotinine. The analytical sample was restricted to normal, overweight, and obese participants. After removing children who were underweight (sex-specific BMI-for-age $<5^{th}$ percentile), 1,484 participants remained. Among them, 132 had at least one missing value for birthweight, breastfed as an infant, poverty ratio, and whether or not they had received food stamps in the past 12 months (an indicator of food insecurity) and were

excluded from the analysis. The remaining 1,352 children constituted the study population.

3.2.3 Study Variables

Outcome- Overweight

The primary outcome of interest in this study was overweight defined by the CDC Growth Charts[14] as a sex-specific BMI-for-age ≥85th percentile. The outcome was dichotomous indicating either overweight or not overweight.

Exposure- Exposure to Environmental Tobacco Smoke (ETS)

The primary exposure was environmental tobacco smoke (ETS) measured by serum cotinine levels. As described previously, serum cotinine is a major metabolite of nicotine and higher levels indicate higher exposure to ETS.[15] While the half-life of nicotine is 2 to 3 hours, the half-life of cotinine is approximately 17 hours. Because of the longer half-life, levels of cotinine remain relatively stable throughout the day making a random cotinine measure a reasonable indicator of daily nicotine exposure.[15] NHANES measured cotinine in the blood of participants ages 3 and older.[10]

In this study, serum cotinine was examined both continuously and also categorically. Typically, non-smokers have serum cotinine measuring <1ng/mL; however, those with heavy second-hand smoke exposure can have serum cotinine levels ranging from 1–10 ng/mL. Active smokers generally have serum cotinine levels >10 ng/mL that can climb upwards of 500 ng/mL.[16] In NHANES, the lower level of detection in the laboratory was 0.015 ng/mL. There were instances in the NHANES data set where an individual's result was below the lower level of detection; in these cases, the serum

cotinine value was recorded as 0.015 ng/mL divided by the square root of 2. In this analysis, serum cotinine was categorized as follows: 0 to <0.015 ng/mL (below level of detection), 0.015 ng/mL to <1 ng/mL (mild to moderate ETS exposure), and ≥1 ng/mL (heavy ETS exposure).

Covariates

Potential confounders including birthweight (grams), maternal smoking during pregnancy (Y/N), age, sex, race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Hispanic, and Other), parental SES (measured by the family monthly poverty level index-ratio of monthly family income to the Department of Health and Human Services poverty guidelines specific to family size), parental education, breastfed (Y/N) and duration of breastfeeding defined by quartiles (0 days, 1–60 days, 61–243 days, and 244+ days) physical inactivity (hours spent watching TV or videos in the past 30 days plus minutes spent in sedentary activity on a typical day: categorized as more than 2 hours a day vs. 2 hours or less), average daily caloric intake and average daily grams of sugar consumed (measured by a 2-day dietary recall), diet quality (measured by the Health Eating Index 2010 (HEI-2010))[17] and food insecurity (family received food stamps in the past 12 months) were explored. Sugar intake (g) and total energy (kcal) were calculated by averaging the total sugar and total energy from each of the two 24-hour recall periods. Nutrient estimates were obtained using the USDA's Food and Nutrient Database for Dietary Studies (FNDDS).[18] Information on demographics, SES, diet, health and medical history was reported on the Sample Person Questionnaire and information for participants <16 was reported by an adult proxy.[8] For children 6–11, diet interviews were conducted

with the child and the assistance of a proxy familiar with their dietary habits. For children under 6, the interviews for survey participants were conducted with a proxy most familiar with their dietary habits.[11, 12] The Healthy Eating Index (HEI) measures overall diet quality. It consists of 12 components: (1) total fruit, (2) whole fruit, (3) total vegetables, (4) greens and beans, (5) whole grains, (6) dairy, (7) total protein foods, (8) seafood and plant proteins, (9) fatty acids, (10) refined grains, (11) sodium, and (12) empty calories. The scores of each component are summed together to create a total score (maximum possible value of 100), with higher values indicating an overall better diet quality. Validation of the HEI-2010 found overall adherence to dietary guidelines to be relatively poor in the NHANES population.[19]

3.2.4 Data Analysis

Descriptive analyses were conducted to examine the distribution and frequencies of the exposure and outcome variables as well as the covariates. Bivariate frequency tables between each potential confounder, outcome, and exposure were examined. Mean and standard deviations were calculated for continuous variables. Serum cotinine values were examined by overweight status (overweight vs. not overweight).

Logistic regression was used to examine both the crude and adjusted measure of association between ETS and overweight. Potential confounders were added to the model one at a time and the relationship with the exposure and outcome was assessed. If the addition of the confounder appreciably altered the measure of association, the confounder was left in the model, otherwise, it was removed. When a final model was selected, the

confounders previously excluded were reexamined before deciding on the final model. Additionally, the subset of the population whose mothers did not smoke during pregnancy was examined in a subgroup analysis. This was done because of the difficulty in distinguishing the health effects of smoking during pregnancy from those occurring after birth, since women who smoked during pregnancy were perhaps likely to continue smoking post-delivery.[20]

Stratified analyses were conducted to examine effect measure modification (EMM) by sex (male vs. female), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Hispanic, and Other), breastfed as an infant (yes/no), and poverty level (below poverty level vs. at or above poverty level). Stratified analyses were only conducted in the subset of the population of children born to mothers who did not smoke during pregnancy.

The sample was analyzed using SAS statistical software (version 9.3, SAS Institute). NHANES survey weights were not taken into account given the specific subsample used. Additionally, the aim was to assess association and not prevalence; therefore, results are specific to this sample.[21, 22]

3.3 RESULTS

Among 1,352 3–6 year olds from NHANES 2007–2012 with a BMI $\geq 5^{th}$ percentile for age and sex, an available serum cotinine lab value, and non-missing values for covariates, 399 (29.5%) children met the criteria for overweight (BMI $\geq 85^{th}$ percentile for age and sex). Demographics are presented by overweight status in **Table 3.1**.

Overweight 3–6 year olds had serum cotinine levels, on average, 0.2 ng/mL greater than normal weight children. Age, dietary intake variables, and the distributions of sex, food insecurity, breastfeeding practice, and maternal smoking during pregnancy were similar between overweight and normal weight children in the study sample.

Table 3.2 shows the distribution of demographic characteristics by serum cotinine category. The percentage of overweight subjects increased as serum cotinine level increased. The proportion of children from food insecure families increased as serum cotinine level increased. A similar pattern was observed such that the prevalence of maternal smoking during pregnancy and the proportion of children who spent more than 2 hours per day engaged with TV/video games increased as serum cotinine increased. The proportion of children who were breastfed and the dietary quality score both decreased as serum cotinine level increased.

Table 3.3 examines the dietary quality of subjects by overweight status. There was little variability in diet quality between overweight and normal weight 3–6 year olds as measured by the HEI-2010 based on two days of dietary recall data. However, overall diet quality for both groups was poor with overweight children scoring 53.1 and normal weight children scoring 53.5 for HEI-2010 total.

Higher levels of serum cotinine were associated with an increased risk of overweight among 3 to 6-year-old children in NHANES III (Cochran-Armitage one-sided trend test, $p=0.02$). After adjusting for age, male sex, race/ethnicity, birthweight category (low: < 2500 g, normal: 2500 to < 4000g, high: ≥ 4000 g), poverty ratio, and breastfed as an infant (Y/N), the positive association remained. In the fully adjusted model with

unexposed as the referent group, the OR (95% CI) for overweight associated with a serum cotinine in the range of 0.015 ng/mL to < 1 ng/mL was 1.11 (0.79, 1.56); and for ≥ 1 ng/mL, it was 1.59 (1.01, 2.52) (**Table 3.4**). There was little variation in energy intake, sugar intake, or diet quality between overweight and normal weight subjects and inclusion of the dietary variables in the model did not appreciably alter the effect estimates; therefore, none of the dietary variables were included in the final model.

When examining the subset of the population who were born to mothers who did not smoke during pregnancy, the effect of ETS was slightly stronger (**Table 3.5**). In the fully adjusted model (adjusted for age, male sex, race/ethnicity, birthweight category, poverty ratio, and breastfed), the OR (95% CI) for serum cotinine measuring 0.015 ng/mL to < 1 ng/mL was 1.18 (0.83, 1.66); and for ≥ 1 ng/mL was 1.49 (0.88, 2.53) compared to children who were unexposed to ETS.

Models stratified by poverty level (**Table 3.6**) showed a stronger effect among those living below the poverty level. However, due to smaller cell sizes, confidence intervals widen and should be interpreted with caution. In the fully adjusted models for those living below the poverty level (adjusted for age, male sex, race/ethnicity, birthweight category, and breastfed), the OR (95% CI) for serum cotinine levels from 0.015 ng/mL to < 1 ng/mL was 1.45 (0.71, 2.97); and for ≥ 1 ng/mL was 2.14 (0.90, 5.09). The effect was much weaker for subjects living at or above the poverty level: the OR (95% CI) for serum cotinine levels of 0.015 ng/mL to < 1 ng/mL was 1.16 (0.79, 1.72); and for ≥ 1 ng/mL was 1.26 (0.57, 2.76).

Models stratified by whether or not the child was breastfed as an infant (**Table**

3.7) showed a stronger effect for children who were not breastfed as infants. In the fully adjusted model for children who were not breastfed as infants (adjusted for age, male sex, race/ethnicity, birthweight category, and poverty ratio) the OR (95% CI) for serum cotinine in the range 0.015 ng/mL to < 1 ng/mL was 1.69 (0.79, 3.63); and for ≥ 1 ng/mL was 2.36 (0.93, 6.00). In contrast, for children who were breastfed as infants, the OR (95% CI) for serum cotinine levels of 0.015 ng/mL to < 1 ng/mL was 1.07 (0.72, 1.57); and for ≥ 1 ng/mL was 1.35 (0.66, 2.74).

Models stratified by sex (**Table 3.8**) showed a measurably stronger effect for females compared to males. In the fully adjusted model for females (adjusted for age, race/ethnicity, birthweight category, breastfed as an infant, and poverty ratio), the OR (95% CI) for serum cotinine levels of 0.015 ng/mL to < 1 ng/mL was 1.42 (0.84, 2.41); and for ≥ 1 ng/mL was 2.06 (0.95, 4.50). In the fully adjusted model for males, the association observed in the crude model was almost entirely explained away by adjusting for confounders. The OR (95% CI) for serum cotinine levels of 0.015 ng/mL to < 1 ng/mL was 1.00 (0.63, 1.58); and for ≥ 1 ng/mL was 1.12 (0.54, 2.33).

Models stratified by race/ethnicity (**Table 3.9**) yielded similar positive results across strata. Due to the small cell sizes, especially in the “other race” group, results should be interpreted cautiously.

3.4 DISCUSSION

In this diverse sample of normal weight, overweight and obese 3–6 year olds, we observed that exposure to environmental tobacco smoke has a positive association with

risk of overweight/obesity, with a dose-response effect observed indicating higher levels of ETS are associated with an increased risk of being overweight/obese. A modest association remained after adjustment for age, sex, race/ethnicity, birthweight, poverty level, and whether the child was breastfed as an infant. Further evaluation of a subpopulation of children born to mothers who did not smoke during pregnancy revealed similar results. The positive dose-response relationship was present among subgroups of children from families living both below and at or above the poverty level, children both breastfed and not breastfed as infants, females, and non-Hispanic whites, Hispanics, and "other race", though in many cases confidence intervals were wider due to small numbers.

Our findings are consistent with other studies that show a positive association between ETS and childhood overweight/obesity.[3–5] We observed a positive-dose response relationship among females that was substantially stronger than that observed among males. Females with higher levels of ETS were more likely to be overweight, whereas there was little to no effect observed for males. This observation is consistent with a study of maternal smoking and fetal growth.[23] The study was conducted in 856 Scandinavian women, where 306 (35.7%) women were non-smokers, 242 (28.3%) were light smokers (1–9 cigarettes per day), and 308 (36.0%) were heavy smokers (≥ 10 cigarettes per day). It was observed that maternal smoking affected birthweight parameters of male and female fetuses differently, with males affected more so than females. Male infants born to mothers who smoked heavily during pregnancy weighed, on average, 316 grams less than males born to mothers who did not smoke during

pregnancy. Females born to mothers who smoked heavily during pregnancy weighed, on average, 177 grams less than females born to mothers who did not smoke during pregnancy. Additionally, among boys, but not girls, a smaller head circumference was observed for male infants born to mothers who smoked heavily during pregnancy compared to mothers who did not smoke during pregnancy.[23] Investigators concluded that tobacco is more detrimental to male compared to female fetuses.[23] The biologic rationale for this conclusion is not well understood. It is worth noting that these authors examined gestational exposure whereas we examined early childhood exposure. However, given the similarities of the differences in effect on male and female offspring, a common biologic mechanism cannot be ruled out.

In a prospective, observational cohort study of 2,055 Canadian children, 58.4% of children were never exposed to household smoking, 33.5% had transient exposure, and 8.1% had continuous exposure.[5] Exposure in that study was defined based on parental report of smokers in the household. Aside from the measurement error and bias associated with self-reported tobacco use, only examining the primary residence as a place of exposure fails to capture other possible exposure locations, including a relative's house, at daycare, a friend's house, etc. There is likely significant misclassification of exposure using these methods. Our study relied on serum cotinine, which objectively measures continuous daily smoke exposure more accurately. We were able to classify a truly non-exposed group as those who had serum cotinine levels below the lower level of detection by the lab. 18.2% of our population fell into this exposure category. We considered serum cotinine levels between 0.015 and <1 ng/mL to have low exposure to

environmental tobacco smoke, which accounted for 65.2% of our population. Lastly, those with serum cotinine levels ≥1 ng/mL were defined as a moderate-to-high level of exposure. An additional strength of this method is that we were able to assess all environmental smoke exposure, not just exposure in the primary residence. With our method, we observed an increase in risk of overweight for children with greater levels of tobacco exposure. Our effect was similar relative to the reference group (**Table 3.5**) as compared to the results of the Canadian study. However, unlike the Canadian study, we observed a dose-response effect.

In the fully adjusted models in the Canadian study, investigators adjusted for gestational smoking, maternal BMI 17 months after birth, birthweight for gestational age, and maternal immigration status. Information on family income, maternal education, and child's soft drink and snack intake over the last week was collected, but these variables were not retained in the final model.[5] We found poverty ratio to have a substantial impact on the magnitude of the effect estimate and therefore, we included it in our final model. We also had information on whether the child was breastfed as an infant, which the Canadian study did not collect. We consider these enhancements a strength of our study lending validity to our results.

In the PIAMA (Prevention and Incidence of Asthma and Mite Allergy) birth cohort study, a prospective study of 1,687 Dutch children born between 1996 and 1997, investigators set out to identify predictors of overweight at age 8.[4] A strong independent predictor of overweight at age 8 was the presences of smokers in the household (OR=1.73; 95% CI: 1.47, 2.04). Other predictors included in the final model were BMI of

father, BMI of mother, birthweight (kg), sex, and hospital as the birth site. Our positive association was not as strong as the effect seen here; however, the PIAMA study did not include maternal smoking during pregnancy in their final model, which we believe may have attenuated their observed effect. Furthermore, using serum cotinine to define exposure, rather than relying on parental report of smoking in the household yields a more reliable exposure assessment and reduces misclassification of average daily exposure, with the added advantage of not limiting the exposure assessment to the primary residence.

The Southern California Children's Health Study (CHS) examined the association between BMI and exposure to secondhand tobacco smoke (SHS) and air pollution among 3,318 fourth graders.[3] Children were followed for prospectively for 8 years. SHS was assessed by parents response to the following questions, "Does anyone living in this child's home currently smoke cigarettes, cigars, or pipes on a daily basis INSIDE THE HOME?" or "In the past, has anyone living in this child's home ever smoked cigarettes on a daily basis INSIDE THE HOME when the child was living there?". If the parent reported the child was exposed currently, a follow up question was asked, "How many people smoke inside this child's home on a daily basis?" with responses 0, 1, or 2 or more smokers. Children exposed to SHS had, on average, a BMI 1.23 kg/m^2 greater than children not exposed to SHS (95% CI: 0.86, 1.61).[3] This study, like many previous studies, classified the exposure based on parental report of smoking in the home. This method of characterizing exposure fails to capture any exposure outside of the primary residence, such as exposure at daycare, other family member's homes, and the homes of

friends. Additionally, in this study, fully adjusted models controlled for ethnicity, sex, community, year of enrollment, and age, but not diet.

There were several unique strengths of this study. Misclassification of exposure (ETS) is unlikely because a biologic marker of tobacco exposure was used (serum cotinine). Previous studies that have relied on parental self-report to estimate the child's ETS exposure likely suffer from misclassification error and/or bias. Underreporting is common in studies where smoking status is self-reported. Additionally, using serum cotinine in our study, we are able to ascertain the average daily ETS exposure, not just exposure in the child's residence. Children 3–6 years old may spend most of their time during the week in daycare or school, where the environment may differ from their household. Using serum cotinine measured in the blood, we are able to capture this. Misclassification of the outcome (overweight/obesity) is also unlikely given that height and weight were measured in NHANES.

We did examine parental self-report of smoking in the household as the primary exposure, rather than serum cotinine, in the form of a sensitivity analysis. Despite a strong association between serum cotinine and number of smokers in the household, and serum cotinine and number of cigarettes smoked daily in the household, we show evidence that this method would lead to misclassification of exposure. Among children in the highest category of serum cotinine exposure (≥1 ng/ml), 38.0% were from households where the parent reported no smokers (of all tobacco types) living in the primary residence and 37.5% were from households where parents reported more than one pack of cigarettes smoked daily, on average. This observation not only encourages researchers

to question the reliability of self-reported ETS exposure, but provides further evidence that assessing exposure in the primary residence alone is not a valid measure of true ETS exposure among children. Additionally, we ran logistic regression models with a categorical variable for number of smokers in the household (0, 1, and 2+) rather than serum cotinine categories and we also ran logistic regression models with a categorical variable for average number of cigarettes smoked per day in the household (0, 1 to <20, 20+). In both cases, these exposure variables failed to show the dose-response effect we observed using objectively measured exposure information. In our sensitivity analysis using self-reported exposure data, the strongest association was observed in the lowest exposure category relative to the reference group. This observation is consistent with the results observed in the Quebec Longitudinal Study of Child Development[5] where a strong effect was observed for transient exposure from birth to age 7, but less so for continuous exposure from birth to age seven.

Finally, we must consider the potentially confounding effects of maternal smoking while pregnant, distinguishing this behavior from current maternal and/or household smoking exposures. A strong, positive association between maternal smoking during pregnancy and childhood overweight/obesity has been established by numerous studies.[24–27] Mothers who smoke during pregnancy are likely to continue smoking after delivery. NHANES collected data on whether the mother smoked during pregnancy. By restricting our analyses to the subgroup of children born to mothers who did not smoke during pregnancy, we were able minimize this potential source of confounding.

One limitation of this study, as is the case with all cross-sectional studies, is that

exposure and disease were assessed at the same time; therefore, we are unable to determine temporality of the observed association. Our observations are strengthened by the findings from the three prospective cohort studies previously discussed that are in agreement with our results.[3–5]

Another potential limitation in this line of research is the potential for residual confounding. Higher levels of smoking are observed among lower socioeconomic status (SES) groups and SES is a strong, well-established risk factor for overweight/obesity. We adjusted for markers of SES including race/ethnicity, birthweight (low, normal, high), ratio of family income to poverty (specific to family size), and whether the child was breastfed as an infant (oftentimes an indicator of increased income, parental education and/or other more general health-related behaviors). Adjusting for these factors attenuated, but did not completely explain the positive association between ETS and overweight/obesity. However, residual confounding cannot be ruled out.

Caution should be exercised when interpreting the results of our stratified models. Due to the small numbers, confidence intervals around the point estimates are wider relative to the estimates for the entire population.

While the biologic mechanism explaining the relationship between ETS and overweight/obesity is not fully understood, there are numerous hypotheses. Several studies have pointed to the inflammatory effects of tobacco smoke and the subsequent detrimental effects on lipid metabolism, insulin resistance, and the nervous system.[3, 5, 28]

In summary, in this diverse sample of 3–6 year olds, we observed a positive association between ETS exposure and overweight/obesity. This study contributes to the

growing body of literature that ETS exposure has detrimental effects on weight and metabolic indicators with implications for the public health problem of childhood obesity. Future studies should evaluate this association prospectively in a larger population.

Table 3.1 Distribution of covariates by weight status among 3–6 year olds in NHANES 2007–2012

	Overweight BMI-for-age ≥85th percentile (n=399)	**Normal Weight 5th≤ BMI-for-age <85th percentile (n=953)**
Age	4.7 ± 1.1	4.6 ± 1.1
Sex		
Male	209 (52.4%)	512 (53.7%)
Female	190 (47.6%)	441 (46.3%)
Child's BMI-for-age percentile	94.8 ± 4.6	50.5 ± 23.3
Race/Ethnicity		
Non-Hispanic White	102 (25.5%)	295 (31.0%)
Non-Hispanic Black	96 (24.1%)	236 (24.7%)
Hispanic	179 (44.9%)	327 (34.3%)
Other	22 (5.5%)	95 (10.0%)
Breastfed as an infant	255 (63.9%)	645 (67.7%)
Birthweight (grams)	3388.9 ± 614.8	3229.0 ± 596.9
Birthweight category		
Low (< 2500g)	27 (6.8%)	97 (10.2%)
Normal (2500 to < 4000 g)	324 (81.2%)	777 (81.5%)
High (≥ 4000 g)	48 (12.0%)	79 (8.3%)
Watch >2 hours of TV/videos per day	130 (33.8%)	280 (30.4%)
Energy (kcal)[a]	1702.1 ± 533.9	1679.7 ± 508.5
Sugar (g)[a]	114.0 ± 43.7	112.8 ± 43.4
Healthy Eating Index 2010 (HEI-2010) Total Score	53.1 ± 11.7	53.5 ± 11.6
Food insecurity[b]	177 (44.4%)	404 (42.4%)
Mother smoked during pregnancy	52 (13.0%)	146 (15.3%)
Poverty ratio[c]	1.5 ± 1.2	1.8 ± 1.4
Serum cotinine (ng/mL)	0.8 ± 2.2	0.6 ± 1.8
Serum cotinine category		
<0.015 ng/mL (lower level of detection)	64 (16.0%)	182 (19.1%)
0.015 to <1 ng/mL	258 (64.7%)	624 (65.5%)
≥1 ng/mL	77 (19.3%)	147 (15.4%)

Continuous variables are presented as mean ± standard deviation and categorical variables are presented as N (%).

[a]Average daily energy and sugar consumption was obtained from a 2-day dietary recall. If a subject was missing one day, a single day of data was used.

[b]Head of household reported receiving food stamp benefits within the past 12 months.

[c]Monthly family income divided by poverty guidelines specific to household size. A value <1 indicates the household is living below the poverty level, a value of 1 indicates living at the poverty level, and a value >1 indicates living above the poverty level. Higher values indicate greater financial security.

Table 3.2 Distribution of covariates by serum cotinine levels among 3–6 year olds in NHANES 2007–2012

	Serum Cotinine < 0.015 ng/ml n= (246)	**Serum Cotinine ≥0.015 and <1 ng/ml n= (826)**	**Serum Cotinine ≥1 ng/ml n= (224)**
Age	4.7 ± 1.1	4.6 ± 1.1	4.5 ± 1.1
Male, n (%)	140 (56.9%)	465 (52.7%)	116 (51.8%)
BMI percentile	59.8 ± 29.4	63.6 ± 28.3	67.3 ± 26.3
Race/Ethnicity			
Non-Hispanic White, n (%)	71 (28.9%)	239 (27.1%)	87 (38.8%)
Non-Hispanic Black, n (%)	30 (12.2%)	209 (23.7%)	93 (41.5%)
Hispanic, n (%)	120 (48.8%)	363 (41.2%)	23 (10.3%)
Other, n (%)	25 (10.2%)	71 (8.1%)	21 (9.4%)
Breastfed as an infant, n(%)	199 (80.9%)	606 (68.7%)	95 (42.4%)
Birthweight (grams)	3294.8 ± 605.1	3300.0 ± 618.4	3162.1 ± 547.0
Birthweight category, n(%)			
Low birthweight (<2500 g)	24 (9.8%)	76 (8.6%)	24 (10.7%)
Normal birthweight (2500 to <4000 grams)	199 (80.9%)	711 (80.6%)	191 (85.3%)
High birthweight (≥4000 g)	23 (9.4%)	95 (10.8%)	9 (4.0%)
Watch more than 2 hours of TV/Videos per day, n (%)	53 (22.3%)	260 (30.7%)	97 (43.7%)
Energy (kcal)[1]	1629.4 ± 472.4	1684.5 ± 516.6	1754.8 ± 552.4
Sugar (g)[1]	107.2 ± 36.8	113.6 ± 43.5	117.7 ±49.4
Healthy Eating Index 2010 (HEI-2010) Total Score	56.8 ± 10.8	53.8 ± 11.5	47.7 ± 10.8
Food insecurity[2], n (%)	38 (15.5%)	379 (43.0%)	164 (73.2%)
Mother smoked during pregnancy, n (%)	3 (1.2%)	93 (10.5%)	102 (45.5%)
Poverty ratio[3], n (%)	2.6 ± 1.6	1.6 ± 1.3	1.0 ± 0.9
Overweight, n (%)	64 (26.0%)	258 (29.3%)	77 (34.4%)

Continuous variables are presented as mean ± standard deviation and categorical variables are presented as N (%).

[1]Average daily energy and sugar consumption was obtained from a 2-day dietary recall. If a subject was missing one day, a single day of data was used.

[2]Head of household reported receiving food stamp benefits within the past 12 months.

[3]Monthly family income divided by poverty guidelines specific to household size. A value <1 indicates the household is living below the poverty level, a value of 1 indicates living at the poverty level, and a value >1 indicates living above the poverty level. Higher values indicate greater financial security.

Table 3.3 Component and total 2-day scores for overweight and normal weight participants, using the Healthy Eating Index 2010 (HEI-2010), NHANES 2007–2012

Component	Max Points	Overweight (BMI-for-age ≥ 85th percentile) Mean (SD)	Normal Weight (5th≤ BMI-for-age< 85th percentile) Mean (SD)
Total vegetables[1]	5	2.1±1.3	2.1±1.3
Greens and beans[1]	5	0.8±1.5	0.9±1.6
Total fruit[2]	5	3.6±1.7	3.5±1.7
Whole fruit[3]	5	3.3±2.0	3.3±2.0
Whole grains	10	2.6±2.6	2.6±2.6
Total dairy[4]	10	8.1±2.4	8.1±2.5
Total protein foods[5]	5	3.9±1.3	3.8±1.2
Seafood and plant proteins[5,6]	5	1.7±1.9	1.9±2.0
Fatty acids[7]	10	3.6±3.0	3.5±2.9
Refined grains	10	5.4±3.0	5.6±3.1
Sodium	10	5.3±2.9	5.6±2.9
Empty calories[8]	20	12.9±4.9	12.6±4.8
HEI-2010 Total Score	100	53.1±11.7	53.5±11.6

*For subjects with only 1 day of recall, the appropriate HEI-2010 SAS code was used to calculate HEI total score.

[1]Includes any beans and peas not counted as Total Protein Foods.

[2]Includes fruit juice.

[3]Includes all forms except juice.

[4]Includes all milk products such as fluid milk, yogurt, cheese, and fortified soy beverages.

[5]Beans and peas are included here (and not with vegetables) when the Total Protein Foods standard is otherwise not met.

[6]Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods.

[7]Ratio of poly- and monounsaturated fatty acids to saturated fatty acids.

[8]Calories from solid fats, alcohol, and added sugars; threshold for counting alcohol is >13 grams/1000kcal.

Table 3.4 Entire Population: The association between Environmental Tobacco Smoke (ETS) and overweight (N=1,352)

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	246	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	882	1.18 (0.85, 1.62)	1.23 (0.89, 1.71)	1.11 (0.79, 1.56)
≥1 ng/ml	224	1.49 (1.00, 2.21)	1.86 (1.22, 2.83)	1.59 (1.01, 2.52)

Model 1 is unadjusted.
Model 2 is adjusted for age, male sex, and race/ethnicity.
Model 3 is adjusted for age, male sex, race/ethnicity (white, black, Hispanic, other), birthweight (low, normal, high), poverty ratio, and breastfed as an infant.

Table 3.5 The association between Environmental Tobacco Smoke (ETS) and overweight among children born to mothers who did not smoke during pregnancy (n=1,154)

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	243	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	789	1.26 (0.91, 1.74)	1.29 (0.93, 1.80)	1.18 (0.83, 1.66)
≥1 ng/ml	122	1.56 (0.97, 2.49)	1.76 (1.07, 2.89)	1.49 (0.88, 2.53)

Model 1 is unadjusted.
Model 2 is adjusted for age, male sex, and race/ethnicity.
Model 3 is adjusted for age, male sex, race/ethnicity (white, black, Hispanic, other), birthweight (low, normal, high), poverty ratio, and breastfed as an infant.

Table 3.6 The association between ETS and overweight among 3–6 year olds in NHANES 2007–2012 stratified by poverty level* (n=432)

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Below the poverty level	432			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	46	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	304	1.32 (0.66, 2.68)	1.47 (0.72, 3.01)	1.45 (0.71, 2.97)
≥1 ng/ml	82	1.72 (0.78, 3.81)	2.19 (0.93, 5.15)	2.14 (0.90, 5.09)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
At or above the poverty level	722			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	197	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	485	1.21 (0.83, 1.76)	1.25 (0.85, 1.83)	1.16 (0.79, 1.72)
≥1 ng/ml	40	1.23 (0.58, 2.59)	1.38 (0.64, 2.99)	1.26 (0.57, 2.76)

*Population where mothers did not smoke during pregnancy.
Model 1 is unadjusted.
Model 2 is adjusted for age, male sex, and race/ethnicity.
Model 3 is adjusted for age, male sex, race/ethnicity (white, black, Hispanic, other), birthweight (low, normal, high), and breastfed as an infant.

Table 3.7 The association between ETS and overweight 3–6 year olds in NHANES 2007–2012 stratified by infant feeding practice*

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Breastfed as infants	808			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	199	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	558	1.19 (0.82, 1.71)	1.19 (0.82, 1.74)	1.07 (0.72, 1.57)
≥1 ng/ml	51	1.45 (0.75, 2.82)	1.61 (0.81, 3.19)	1.35 (0.66, 2.74)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Not breastfed as infants	346			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	44	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	231	1.39 (0.68, 2.84)	1.59 (0.76, 3.32)	1.69 (0.79, 3.63)
≥1 ng/ml	71	1.54 (0.68, 3.50)	2.09 (0.87, 5.03)	2.36 (0.93, 6.00)

*Population where mothers did not smoke during pregnancy.
Model 1 is unadjusted.
Model 2 is adjusted for age, male sex, and race/ethnicity.
Model 3 is adjusted for age, male sex, race/ethnicity (white, black, Hispanic, other), birthweight (low, normal, high), and poverty ratio.

Table 3.8 The association between ETS and overweight for 3–6 year olds in NHANES 2007–2012 stratified by sex*

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Males	614			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	139	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	412	1.11 (0.72, 1.70)	1.08 (0.70, 1.68)	1.00 (0.63, 1.58)
≥1 ng/ml	63	1.24 (0.65, 2.36)	1.32 (0.66, 2.64)	1.12 (0.54, 2.33)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Females	540			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	104	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	377	1.48 (0.90, 2.43)	1.57 (0.94, 2.61)	1.42 (0.84, 2.41)
≥1 ng/ml	59	2.02 (1.01, 4.02)	2.39 (1.16, 4.93)	2.06 (0.95, 4.50)

*Population where mothers did not smoke during pregnancy.
Model 1 is unadjusted.
Model 2 is adjusted for age, and race/ethnicity.
Model 3 is adjusted for age, race/ethnicity (white, black, Hispanic, other), birthweight (low, normal, high), breastfed as an infant, and poverty ratio.

Table 3.9 The association between ETS and overweight among 3–6 year olds in NHANES 2007–2012 stratified by race/ethnicity*

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Non-Hispanic Whites	397			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	71	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	239	1.28 (0.64, 2.57)	1.21 (0.60, 2.44)	1.11 (0.52, 2.34)
≥1 ng/ml	87	1.84 (0.71, 4.79)	1.74 (0.66, 4.57)	1.65 (0.55, 4.94)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Hispanics	506			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	120	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	363	1.02 (0.66, 1.58)	1.07 (0.69, 1.67)	1.06 (0.68, 1.66)
≥1 ng/ml	23	2.27 (0.87, 5.91)	2.40 (0.92, 6.31)	2.30 (0.87, 6.13)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Non-Hispanic Blacks	322			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	30	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	209	1.82 (0.71, 4.68)	1.77 (0.68, 4.57)	1.46 (0.53, 4.00)
≥1 ng/ml	89	1.78 (0.63, 5.02)	1.71 (0.60, 4.86)	1.28 (0.40, 4.07)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Other	117			
Serum Cotinine				
Below level of detection (<0.015 ng/ml)	25	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0.015 to <1 ng/ml	71	3.43 (0.72, 16.36)	3.58 (0.74, 17.30)	2.40 (0.44, 13.13)
≥1 ng/ml	21	11.50 (1.01, 131.28)	13.20 (1.10, 158.29)	8.13 (0.53, 124.86)

*Population where mothers did not smoke during pregnancy.
Model 1 is unadjusted.
Model 2 is adjusted for age, and race/ethnicity.
Model 3 is adjusted for age, male sex, birthweight (low, normal, high), breastfed as an infant, and poverty ratio.

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4. THE ASSOCIATION BETWEEN MATERNAL EXPOSURE TO ANTIBIOTICS DURING PREGNANCY AND SMALL FOR GESTATIONAL AGE

4.1 INTRODUCTION

Antibiotics are one of the most frequently used drugs during pregnancy. The most common indications for use include respiratory infection, urinary tract infection, vaginal/yeast infection, and skin infection.[1] However, recent research suggests that antibiotic use during pregnancy may alter the microbial diversity of the gastrointestinal (GI) tract not only of the mother but also of the baby.[2,3] Altered gut microbiota in utero and infancy may increase the susceptibility of these infants to obesity later in childhood.[4] This is supported by several studies that showed differences in fecal microbiota in obese compared to lean and normal weight individuals.[4–7] Kalliomaki, et al. also reported that 40% of overweight children and 13% of normal-weight children were exposed to antibiotics in the first 6 months of life, although their sample size was small (N=49).[4] Recent studies that examined antibiotic use in pregnancy suggested an association with infant birthweight. However, studies on maternal antibiotic use during pregnancy and infant birthweight have yielded conflicting results and comparisons across studies are restricted by inconsistencies in defining the outcome, different methods and timing of exposure assessment, and inability to explore various characteristics of exposure including duration of use, trimester of use, class of antibiotic, and indication for use.

Two studies have reported higher birthweight among infants born to mothers who used antibiotics during pregnancy.[8, 9] In a study of 38,151 women who gave birth to infants without congenital abnormalities, 6,554 (17.2%) of women self-reported

antibiotic use during pregnancy, with 5,518 (84.2%) of these women reporting penicillin class antibiotics.[8] Women who reported antibiotic use during pregnancy gave birth to infants who were, on average, 36 grams heavier than infants born to mothers who did not report antibiotic use (3311 g (SD=510) vs. 3275 g (SD=521)). However, the objectives of this study were to evaluate the frequency of, and indications for, antibiotic use during pregnancy. Intrauterine infection is associated with preterm delivery, which is strongly correlated with birthweight. Thus, the association with higher birthweights might be due to preventing preterm delivery.[10]

Another study of primiparous women with live births or still births after 28 weeks gestation in North Jutland, Denmark, reported that mothers who redeemed any prescription for amoxicillin during pregnancy gave birth to infants who weighed, on average, 57 grams more at birth than infants born to mothers who did not redeem any prescriptions in the exposure period ranging from 3 months prior to pregnancy through to the end of pregnancy.[9] Models were adjusted for maternal age, gestational age, and maternal smoking. When restricting to full term births, amoxicillin use during pregnancy was also protective against low birthweight (OR=0.63; 95% CI: 0.26, 1.53).

Other studies have observed an association between antibiotic use during pregnancy and lower birthweight.[2,11] In the Newborn Epigenetic STudy (NEST), a prospective cohort study of women and children, infants born to mothers who used antibiotics during pregnancy, on average, weighed 133 grams (se=50.70) less than infants born to mothers who did not take antibiotics during pregnancy.[2] Similarly, in a study of 2,128 pregnant women from the pre-birth Project Viva cohort, antibiotic use during

pregnancy was associated with lower BW/GA-z scores; BW/GA-z scores were one-ninth of a standard deviation lower for infants exposed to antibiotics in utero compared to unexposed infants (β= -0.11; 95% CI: -0.20, -0.01). The NEST study was unable to examine duration of antibiotic use and both studies were unable to rule out confounding by indication.[2, 11] It is possible that the lower observed birthweight is due, in part, to the indication for which the antibiotics were prescribed.

Further research on the relationship between maternal antibiotic use during pregnancy and birthweight is needed. Only a limited number of studies have been conducted on this topic, and the results have been conflicting. One possible reason for these discrepant results could be confounding by indication. In other words, the relationships previously observed between maternal antibiotic use and birthweight may actually be driven by the indication for which the antibiotic was prescribed. Additionally, characteristics of maternal antibiotic use during pregnancy including dose, duration, and timing of use are understudied. Understanding how maternal antibiotic use during pregnancy is related to birthweight can have important public health implications including prevention of low (and possibly high) birthweight and subsequent childhood obesity as well as targeted intervention strategies.

4.2 METHODS

4.2.1 Data Source

The study population consisted of control participants from the Slone Epidemiology Center's Birth Defects Study (BDS).[12] The BDS interviewed mothers of

infants born with major structural birth defects ascertained from birth and tertiary care hospitals in Boston, Philadelphia, Toronto, Iowa, Tennessee, New York, and San Diego. For the present analysis, mothers of control subjects who were interviewed between 1998 and 2015 were included. They were ascertained from birth hospitals where cases were ascertained, except in Massachusetts where a random sample of births were identified from vital records information provided by the Massachusetts Department of Public Health (MDPH).[13] All subjects were identified within five months of delivery and all mothers were interviewed within six months of delivery.

In the Birth Defects Study, data were available on characteristics of maternal antibiotic use during pregnancy including timing, dose, duration, indication, and class of antibiotic used. Mothers were interviewed by study nurses using standardized questionnaires with specific prompts for antibiotics and infections. Computer assisted telephone interviews were conducted by trained nurses. In addition to medication and vitamin use, detailed questions were also asked about demographic, reproductive, and behavioral factors.[12]

4.2.2 Study Population and Design

Included in this retrospective cohort analysis are 11,421 control subjects from the Birth Defects Study. We restricted our analysis to 11,077 singleton births (97% of births) and excluded another 21 subjects with missing values for infant birthweight. A complete case analysis was conducted, removing subjects with missing information on maternal variables: age, race, smoking during pregnancy, education, height, and weight. A final

sample size of 10,647 subjects was used for this analysis. Among them, approximately 2,677 (25.1%) reported maternal antibiotic use during pregnancy.

*4.2.*3 *Study Variables*

Outcome- Small for Gestational Age (SGA)

The primary outcome was small for gestational age (SGA). Infant birthweight was self-reported by the mother at the time of interview. The gestational age (in weeks) was calculated by subtracting the date of the last menstrual period (LMP) from the delivery date and dividing by 7. Infants with birthweight-for-gestational-age z-scores below the 10^{th} percentile as defined according to the growth curves developed by Oken, et al. were classified as SGA.[14] These growth curves used data from the United States Natality datasets and analyzed nearly 7 million infants (6,690,717) born at 22–44 weeks gestation between 1999 and 2000 to US residents. We chose to use SGA as our outcome rather than birthweight with adjustment for gestational age for two reasons: (1) adjusting for gestational age assumes a strict linear relationship between birthweight and gestational age, which is not necessarily the case, and 2) even though gestational age is highly correlated with birthweight, it is in the causal pathway between antibiotic use and birthweight (or a consequence of birthweight) and therefore adjustment can create biased estimates.[14] We also examined birthweight adjusted for gestational age z-score (BW/GA-z) as a continuous measure.

Exposure-Maternal Antibiotic Use during Pregnancy

Women were asked if they had taken any drugs for a list of illnesses, including respiratory, genitourinary and other infections, and if they had taken any of a list of specific medications [**Appendix 1**]. Women who couldn't remember the exact name of the antibiotic were read a list to aid recall [**Appendix 2**]. For each reported medication, women were asked to state the drug name, start and stop dates, and reason for taking it. The primary exposure was maternal antibiotic use during pregnancy, defined as a dichotomous variable (yes/no), regardless of the number of uses. Other characteristics of antibiotic exposure that were considered included trimester of use, class of antibiotic, duration of use (0 days, 1–6 days, 7–10 days, 11+ days), and indication for use. Antibiotic class was determined by a research pharmacist based on the reported name of the product and American Hospital Formulary (AHFS)[15] as shown in **Appendix 3**. Classes with at least 90 reports in our dataset were examined, including penicillins, macrolides, cephalosporins, and miscellaneous antibacterials. Additionally, we examined as a group the large number of antibiotics with class not otherwise specified (Antibiotics-NOS). A complete list of indications for antibiotic use is provided in **Appendix 4.** The most common indications for use in the BDS control cohort were respiratory infections and genitourinary infections[1]; therefore, indications for use were classified into three categories: (1) respiratory infections, (2) genitourinary infections, and (3) other. Duration of use was recorded as total time in days the drug was taken over the course of pregnancy, and was examined as a categorical variable with four categories: 0 days, 1–6 days, 7–10 days, and 11+ days. These categories were confirmed by examining tertiles of

duration.

Covariates

Potential confounders included maternal age, maternal pre-pregnancy BMI, maternal smoking status during pregnancy (yes/no), highest level of parental education (less than high school, high school, some college, college plus), and mother's race/ethnicity. Maternal age, pre-pregnancy weight and weight at delivery, smoking status during pregnancy, race/ethnicity, and parental education were all self-reported by the mother during the interview.

4.2.4 Data Analysis

These data were analyzed using SAS statistical software (version 9.3, SAS Institute). Distributions of covariates were examined by SGA status and antibiotic exposure status. Bivariate frequency tables between each potential confounder, outcome, and exposure were examined. Means and standard deviations were calculated for continuous variables.

Multivariable logistic regression was used to examine the adjusted measure of association between maternal antibiotic use during pregnancy and SGA. Multivariable linear regression was used to examine the adjusted measure of association between BW/GA-z and maternal antibiotic use during pregnancy. Potential confounders were added to the model one at a time and the relationship with the exposure and outcome was assessed. If the addition of the confounder altered the measure of association by at least 3%, the confounder was left in the model, if not, it was removed. When a final model was

selected, the confounders previously excluded were reexamined before deciding on the final model. Because maternal smoking is a known risk factor for decreased birthweight, it was retained in all models. Models stratified by maternal smoking during pregnancy and infant sex were examined to explore whether there was evidence of effect measure modification (EMM) by smoking status or infant sex.

The association between SGA and trimester of antibiotic use was also examined. Indication variables for each trimester were included in the model indicating whether the infant was exposed to antibiotics during that trimester. In a sensitivity analysis, we explored these same models restricted to infants only exposed to antibiotics during a single trimester to better isolate trimester-specific effects.

Duration of antibiotic use (in days) was summed across the entire pregnancy for women with multiple episodes of use. Duration was categorized based on approximate tertiles of the data (1–6 days, 7–10 days, and 11+ days; with 0 days as the reference group).

Antibiotic class was also explored. First, we examined the association between SGA and penicillin class antibiotics during pregnancy regardless of whether they reported use of other antibiotic classes. If they used antibiotics, but not penicillin class, they were classified as a ‘Non-penicillin user’. The reference group consisted of mothers who did not report any antibiotic use during pregnancy. This process was repeated for cephalosporin use, macrolide use, antibiotic NOS, and miscellaneous antibacterial use.

We also examined the association between indication for antibiotic use and SGA among mothers who reported antibiotic use during pregnancy. Indication was classified

as respiratory infection if the subject reported respiratory infection at all during pregnancy; otherwise, they were classified as ‘Other Infection’. This same process was repeated for genitourinary infections. Additionally, we explored indication in all subjects using mothers who did not report any antibiotic use during pregnancy as the reference group.

4.3 RESULTS

Among the 10,647 singleton births with available information on maternal antibiotic use during pregnancy and non-missing values for covariates, 891 (8.4%) were classified as SGA. The distributions of demographic variables are presented by SGA status in **Table 4.1**. A slightly smaller percentage of SGA infants were exposed to antibiotics during pregnancy compared to non-SGA infants (23.3% vs. 25.3%). Mothers of SGA infants were younger (mean age: 27.5 vs. 29.3 years), gained less weight during pregnancy (29.1 lbs. vs. 33.1 lbs.), were more likely to be non-white (non-white: 49.6% vs. 28.6%), less likely to be college educated (college or more: 42.8% vs. 59.1%), more likely to have smoked during pregnancy (12.9% vs. 6.5%), and less likely to be overweight/obese (28.4% vs. 34.7%) prior to pregnancy. Gestational age and infant sex were similar across SGA status.

Table 4.2 shows the distributions of covariates by exposure status. More than one in four mothers (n=2,677 (25.1%)) took antibiotics during pregnancy. Infants born to mothers who took antibiotics during pregnancy were less likely to be SGA (7.8% vs. 8.6%); more likely to be female (52.4% vs. 50.1%), born preterm (6.8% vs. 6.0%), born to a smoker (9.4% vs. 6.3%), born to a mother who was obese prior to pregnancy (15.9%

vs. 12.7%); and less likely to be born to a college educated mother or father (53.9% vs. 59.1%). Gestational age, maternal age, maternal weight gain during pregnancy, and maternal race/ethnicity were similar across exposure categories. Birthweight was also similar among infants born to mothers who took antibiotics during pregnancy and those who did not (3398 g vs. 3407 g).

Characteristics of antibiotic use during pregnancy are examined in **Table 4.3**. Of the 2,677 (25.1%) of mothers who took antibiotics during pregnancy, 2,044 (76.4%) had a single exposure period to antibiotics during pregnancy and 633 (23.6%) had 2 or more exposure periods over the course of pregnancy. There were 496 subjects with uncertain exposure periods and, therefore, not classified under any trimester. Of the 2,181 subjects who were able to recall the exposure period, a total of 1,074 (49%) indicated antibiotic use during the first, 669 (31%) in the second and 685 (31%) in the third trimester. A total of 56 subjects reported antibiotic use during pregnancy but had missing values for duration. Median duration of use was 8 days. Of the 2,621 reports for duration, 734 (28.0%) were 1–6 days, 1,130 (43.1%) were 7–10 days, and 757 (28.9%) were >10 days. With respect to antibiotic type, nearly half (47.9%) of reports were classified as antibiotic NOS (not otherwise specified); the mother was unable to recall the type of antibiotic she took. Penicillin was the most common class of antibiotics taken among women who could reliably report drug type (25.6% of reports), followed by macrolides (15.3% of reports), miscellaneous antibacterials (3.0% of reports), cephalosporins (2.6% of reports), and lastly, other antibiotic (5.6%). The most common indication for use was respiratory infections (43.0% of reports), followed by genitourinary infections (37.5% of reports),

and “other infections” (19.5% of reports).

After adjusting for maternal smoking during pregnancy and parental education, any antibiotic use during gestation was associated with a slightly lower risk of SGA (OR=0.86; 95% CI: 0.73, 1.01) (**Table 4.4**). Models stratified by maternal smoking during pregnancy did not reveal evidence of effect measure modification (smokers: OR=0.81, 95% CI: 0.53, 1.25; non-smokers: OR=0.86, 95% CI: 0.72, 1.03) (**Table 4.5**). Models stratified by infant sex revealed nearly identical associations for female and male infants (females: OR=0.87, 95% CI: 0.69, 1.09; males: OR=0.85, 95% CI: 0.67, 1.07) (**Table 4.6**).

As a sensitivity analysis, we explored models with birthweight in grams as a continuous outcome adjusted for gestational age. The fully adjusted model indicated infants exposed to antibiotics during gestation weighed, on average, 12.27 g (95% CI: -6.99, 31.53) more at birth compared to unexposed infants (**Table 4.7**). Fully adjusted models stratified by preterm status (<37 weeks vs. ≥ 37 weeks) indicated a negative association for preterm infants (β= -6.46 g, 95% CI: -89.84, 76.92) and a positive association for full term infants (β=14.44 g, 95% CI: -5.23, 34.12), but the magnitude of these associations were small and the confidence intervals were wide and therefore, should be interpreted with caution (**Table 4.7**).

Fully adjusted multivariable regression models examining the association between maternal antibiotic use during pregnancy and the continuous measure, BW/GA-z, indicated little association (β= 0.03; 95% CI: -0.01, 0.07) (**Table 4.8a**). Models stratified by maternal smoking during pregnancy also indicated no association; however, the

stratum-specific coefficients went in opposite directions (smoker: β= -0.02; 95% CI: -0.17, 0.12; non-smoker: β= 0.03; 95% CI: -0.01, 0.07) (**Table 4.8b**).

Fully adjusted models examining timing of antibiotic use suggested virtually no association between antibiotic use during the first or second trimester and SGA (first trimester: OR=0.93, 95% CI: 0.74, 1.17; second trimester: (OR=1.01, 95% CI: 0.76, 1.33). Antibiotic use during the third trimester was associated with a reduced risk of SGA: (OR= 0.82, 95% CI: 0.61, 1.11) (**Table 4.9a**). Sensitivity analysis restricted to single trimester users revealed nearly identical results for the first and second trimesters (OR=0.92, 95% CI: 0.71, 1.19; second trimester: OR=0.98, 95% CI: 0.71, 1.34). The reduction in risk of SGA was more notable for third trimester users when restricted to single trimester users (OR=0.73, 95% CI: 0.51, 1.04) (**Table 4.9b**).

Models examining the association of duration of antibiotic use on SGA did not show a dose-response effect; there was no increase in the protective effect with increasing duration of use. In fact, the largest protective effect was observed among the shortest duration users relative to non-users (OR=0.70; 95% CI: 0.52, 0.96) (**Table 4.10**). Analysis exploring class of antibiotic revealed no difference in risk of SGA for penicillin class users compared to non-antibiotic users during pregnancy (**Table 4.11a**). Macrolide users had a reduction in risk (OR=0.65; 95% CI: 0.42, 1.01) compared to non-users; cephalosporin users also had a reduction in risk compared to non-users (OR=0.45; 95% CI: 0.14, 1.42). A small reduction in risk was also observed for the antibiotic NOS group (OR=0.87; 95% CI: 0.71, 1.07) as well as for the miscellaneous antibacterial users (OR=0.69; 95% CI: 0.30, 1.60) (**Table 4.11a**) compared to non-users. After adjusting for

other classes, the reduction in risk remained for macrolides users (OR=0.73, 95% CI: 0.42, 1.29) and cephalosporin users (OR=0.50; 95% CI: 0.15, 1.64) compared to non-users (**Table 4.11b**).

Analysis of indication for antibiotic use among antibiotic users revealed that mothers who took antibiotics during pregnancy for respiratory infections were less likely to give birth to SGA infants compared to mothers who took antibiotics during pregnancy for other indications (OR=0.80; 95% CI: 0.60, 1.08) (**Table 4.12a**) and less likely to give birth to SGA infants compared to mothers who reported no antibiotic use during pregnancy (OR=0.76; 95% CI: 0.60, 0.97) (**Table 4.12b**). Conversely, mothers who took antibiotics during pregnancy for genitourinary infections were slightly more likely to give birth to SGA infants compared to mothers who took antibiotics during pregnancy for other indications (OR=1.18; 95% CI: 0.88, 1.59) (**Table 4.12a**); however, the risk of giving birth to and SGA infant was virtually the same when compared to mothers who reported no antibiotic use during pregnancy (OR=0.93; 95% CI: 0.74, 1.16) (**Table 4.12b**).

Antibiotic class, duration, and indication for use were examined in a model together in order to adjust for the effects of the other, with no use at any time in pregnancy as the reference category. Given the small number of reports, in these models, cephalosporin class antibiotics were group with ‘other antibiotic’. These models were stratified by trimester, and for subjects with multiple exposures in a single trimester, only the first was considered. In the first trimester, a notable difference after adjustment for other characteristics of exposure was that short and long duration (1–6 days; 11+ days)

were associated with an increase in risk of SGA compared to women who did not report any antibiotic use during pregnancy (OR=1.62; 95% CI: 0.95, 2.77; OR=1.75; 95% CI: 0.94, 3.26) (**Table 4.13a**). Using antibiotics for genitourinary infections in the second trimester was associated with an increased risk for SGA (OR=1.94; 95% CI: 0.97, 3.87) compared to non-users (**Table 4.13b**), whereas genitourinary infections were associated with a reduction of risk for SGA in the first trimester (OR=0.94; 95% CI: 0.54, 1.64) (**Table 4.13a**). Lastly, in the third trimester, duration of use (1–6 days) was associated with a reduction in risk of SGA compared to mothers who reported no antibiotic use during pregnancy (OR=0.70; 95% CI: 0.36, 1.36). Mothers who reported antibiotic use for genitourinary infections had over a two-fold increase in risk of giving birth to an SGA infant (OR=2.26; 95% CI: 1.07, 4.76) compared to mothers who did not report antibiotic use during pregnancy (**Table 4.13c**).

4.4 DISCUSSION

In this large sample of 10,647 singleton infants from the BDS, we observed that antibiotic use during pregnancy was associated with a reduction in risk of SGA. Stratified analysis revealed this association was similar across maternal smoking status during pregnancy and infant sex. Further analysis revealed antibiotic use during pregnancy had little association with birthweight adjusted for gestational age z-score. Analysis examining timing of antibiotic exposure revealed no association between antibiotic use and SGA in the first and second trimesters. Antibiotic use during the third trimester was associated with a reduction in risk of SGA. A dose-response relationship with duration was not

observed. Penicillin class use was nearly equivalent to no antibiotic use during pregnancy in terms of SGA risk, while macrolides and cephalosporin class use were each associated with a reduction in risk of SGA compared to no use during pregnancy. In analysis stratified by trimester, using antibiotics for 1–6 days in the third trimester was associated with a reduction in risk of SGA and using antibiotics to treat a genitourinary infection in the third trimester was associated with an increase in risk of SGA.

Previous studies examining the association between antibiotic use during pregnancy and birthweight have found conflicting results. These conflicting results may be explained, in part, by different definitions of the outcome, timing of exposure, reference group, and sample size. One study of 2,128 pregnant women from the Project Viva cohort found that antibiotic use during pregnancy was associated with lower BW/GA-z (β= -0.11, 95% CI: -0.20, -0.01)[11], calculated using the growth curves developed by Oken, et al.[14] In a sensitivity analysis, we examined the association between antibiotic use during pregnancy and BW/GA-z, calculated using the same growth curves[14] and found no association. There were many similarities between our study and the study by Mueller, et al. (Project Viva cohort). Both were observational cohort studies and both restricted analyses to singleton births. Study population demographics were also quite similar, specifically with regards to the proportion of each sample that was white, average maternal age and pre-pregnancy BMI, proportion of college educated parents, gestational age at delivery, and proportion of mothers who took antibiotics during pregnancy. One possible explanation for our different conclusions could be misclassification of exposure and outcome in our study. In the Project Viva

cohort, antibiotic use during pregnancy was obtained through electronic medical records (EMR) and birthweight was obtained through hospital medical records.[11] In our study, antibiotic use during pregnancy and birthweight were self-reported by the mother. It is possible that mothers of SGA infants differentially recalled antibiotic exposure during pregnancy, therefore, possibly overstating the observed protective effect of antibiotics on SGA in our study. Other differences included our larger study population (N=10,647 vs. N=2,128) and geographical diversity. Our study includes women from Boston, New York, Philadelphia, Toronto, Iowa, Tennessee and San Diego, whereas the Project Viva cohort was exclusively drawn from Massachusetts. It is possible this relationship could have geographic variability.

Mueller, et al. observed a reduction in BW/GA-z score among antibiotic users compared to non-users (β= -0.11, 95% CI: -0.20, -0.01) that was driven by second trimester use (β= -0.23, 95% CI: -0.37, -0.08).[11] Although authors in the Project Viva Study did observe a statistically significant result, it is important to bring this into the context of clinical significance. Overall, infants exposed to antibiotics in utero had BW/GA-z scores about one-ninth of a standard deviation lower than those of infants not exposed to antibiotics in utero. Infants exposed to antibiotics in the second trimester had BW/GA-z scores about one-fourth of a standard deviation lower than those of infants not exposed to antibiotics in utero.

Another study using birthweight as a continuous outcome measure observed that infants born to mothers who used antibiotics during pregnancy weighed, on average, 42.1 grams (SE=37.4) less at birth compared to mothers who did not use antibiotics during

pregnancy; however, this effect size is only approximately 0.09 lbs.[1] As a sensitivity analysis, we examined birthweight (in grams) as a continuous outcome measure and adjusted for gestational age (in weeks) in our fully adjusted model (**Table 4.7**). We, in fact, observed a slight increase in birthweight for antibiotic users compared to non-users. We opted against using birthweight as a continuous outcome measure and adjusting for gestational age because this method assumes a linear relationship between birthweight and gestational age, and considering it like a confounder violates causal theory.[14] A few key differences in our study populations could explain the conflicting results between our study and that by Nelson, et al. The distribution of antibiotic class was different; we had nearly half of mothers unable to accurately recall the antibiotic they took during pregnancy, whereas Nelson, et al. only reported 19%.[1] This could reduce the precision of our estimates for specific antibiotic classes because some of the subjects truly exposed to a given class are appearing in the antibiotic-NOS group.[1]

The NEST study, a prospective cohort study, examined 396 full term (gestational age $\geq$ 37 weeks) infants and evaluated the association between maternal antibiotic use during pregnancy and infant birthweight. Authors observed that infants exposed to antibiotics in utero weighed, on average, 133 g (SE=50.70) less at birth compared to infants not exposed to antibiotics in utero; however, this effect size is roughly equivalent to 0.3 lbs.[2] We observed virtually no association in our sensitivity analysis examining birthweight adjusted for gestational age. As a further sensitivity analysis, we restricted our population to full term infants, as was done in the NEST study[2], and still observed no association between antibiotic use during pregnancy and birthweight. In our study, we

examined antibiotic use throughout the course of pregnancy, while the NEST study only examined exposure up to 30 weeks of gestation.[2] We did observe that 1–6 days of use (compared to no use at all during pregnancy) in the first trimester of pregnancy was associated with an increase in risk of SGA. Conversely, 1–6 days of use in the third trimester (which is mostly excluded in the NEST study by restricting the exposure window to the first 30 weeks) was associated with a reduction in risk of SGA. It is likely our different exposure periods explain some of the differences observed between our studies. Both our study and the NEST study relied on self-report of maternal antibiotic use during pregnancy, however, our study relied on maternal self-report for birthweight as well, whereas the NEST study obtained information on infant birthweight from medical records.[2] Other differences include our larger sample size (N=10,647 vs. N=396), and greater prevalence of exposure (25.1% vs. 20.7%).

Another study of 10,638 Danish women found that exposure to amoxicillin during gestation was associated with a reduction in risk of low birthweight (<2500 grams) (OR=0.63; 95% CI=0.26, 1.53) compared to infants not exposed to any prescription medication during pregnancy (including antibiotics) in their analysis restricted to full term infants (≥ 37 weeks gestational age).[9] In our analysis, we observed a reduction in risk of SGA for penicillin users compared to antibiotic non-users for the first and second trimesters of pregnancy, although the magnitude of our protective effect was smaller. Jepsen, et al. compared amoxicillin class users to non-users of any prescription medications of any kind throughout pregnancy, rather than just antibiotic non-users, as we did. For this reason, authors of the study acknowledged that the effect of amoxicillin

observed could be inflated.[9]

There were numerous strengths of our study. We had a large, racially and ethnically diverse study population, representative of multiple geographic regions across the U.S. and Canada. We also had information on maternal antibiotic use throughout the entire duration of pregnancy. Information was available on various characteristics of antibiotic exposure including trimester of use, duration of use, class of antibiotic, indication for use. Many prior studies lacked information on these exposure characteristics and cited this as an important limitation. We were also able to examine the outcome in various ways. We used SGA as our primary outcome, but also examined birthweight (g), birthweight (g) adjusted for gestational age (weeks), birthweight stratified by term (preterm: <37 weeks; full-term: ≥ 37 weeks), and BW/GA-z. Finally, data were available on numerous potential confounders including maternal smoking status during pregnancy and parental education.

Our study was not without limitations. Both our exposure, maternal antibiotic use during pregnancy, and our outcome, SGA, relied on self-report by the mother. Infant birthweight has been shown to be recalled reliably by mothers.[16] However, recall accuracy with respect to details regarding antibiotic use (trimester of use, indication for use, duration of use, class of antibiotic used) may be poor. We also cannot rule out residual or unmeasured confounding. Additionally, we did not control for use of other prescription medications during pregnancy, which could have an association with birthweight. We also were unable to fully control for confounding by indication as we did not have information on infections for mothers who did not report antibiotic use during

pregnancy.

In summary, we observed a reduction in risk of SGA for mothers who reported antibiotic use during pregnancy. Results varied by trimester of use, class of antibiotic use, and indication for antibiotic use. Future studies should evaluate this relationship prospectively and rely on prescription databases and electronic medical records to eliminate the potential for recall bias and measurement error. Additionally, future prospective studies should examine if this association is modified by probiotic use during pregnancy. Understanding the effect maternal antibiotic use during pregnancy has on infant birthweight and SGA, and determining if this is the result of antibiotics altering the infant gut microbiota, has important public health implications. If there is a true causal association between maternal antibiotic use and birthweight, this should factor into a physician's decision when evaluating the risks and benefits of prescribing antibiotics to an expectant mother.

Table 4.1 Distribution of covariates by SGA* (Small for Gestational Age) for singleton infants in the Birth Defects Study (BDS) control population

	SGA (n=891)	Not SGA (n=9,756)
Infant exposed to antibiotics during gestation		
Yes	208 (23.3%)	2,469 (25.3%)
Infant sex		
Male	456 (51.2%)	4,792 (49.1%)
Female	435 (48.8%)	4,964 (50.9%)
Gestational age (weeks)	39.1 ± 1.8	39.4 ± 1.7
Preterm (<37 weeks)	75 (8.6%)	582 (6.0%)
Birthweight (g)	2,606 ± 337	3,477 ± 487
BW/GA z-score[a]	-1.7 ± 0.3	0.2 ± 0.8
Mother's age	27.5 ± 6.4	29.3 ± 5.7
Mother's pre-pregnancy BMI	23.9 ± 5.4	24.6 ± 5.3
Mother's pre-pregnancy weight status[b]		
Underweight	72 (8.1%)	429 (4.4%)
Normal weight	566 (63.5%)	5,942 (60.9%)
Overweight	144 (16.2%)	2,059 (21.1%)
Obese	109 (12.2%)	1,326 (13.6%)
Maternal weight gain during pregnancy (lbs)	29.1 ± 16.5	33.1 ± 15.1
Maternal weight gain during pregnancy[c]		
Less than recommended	290 (32.6%)	1,813 (18.6%)
Recommended	250 (28.1%)	2,811 (28.8%)
More than recommended	351 (39.4%)	5,132 (52.6%)
Mother smoked during pregnancy	115 (12.9%)	637 (6.5%)
Mother's race/ethnicity		
White	449 (50.4%)	6,964 (71.4%)
Black	145 (16.3%)	819 (8.4%)
Asian	95 (10.7%)	572 (5.9%)
Nat Am/PacIsl/Other/Multi	120 (13.4%)	844 (8.6%)
Hispanic	82 (9.2%)	557 (5.7%)
Highest parental education level		
Less than high school	78 (8.7%)	438 (4.5%)
High school degree	249 (28.0%)	1,648 (16.9%)
Some college	183 (20.5%)	1,901 (19.5%)
College plus	381 (42.8%)	5,769 (59.1%)

*SGA defined using the growth curves developed by Oken (SGA<10th percentile for weight)[14]

[a]Birthweight- for-gestational age z-score; calculated using the growth curves developed by Oken.[14]

[b]Using mother's pre-pregnancy BMI.

[c] Using IOM 2009 Guidelines.[17]

Table 4.2 Distribution of covariates by exposure to antibiotics during gestation for infants in the Birth Defects Study (BDS) control population

	Exposed to Antibiotics During Gestation (n=2,677)	Not Exposed to Antibiotics During Gestation (n=7,970)
Gestational size[a]		
SGA	208 (7.8%)	683 (8.6%)
Infant sex		
Male	1,274 (47.6%)	3,974 (49.9%)
Female	1,403 (52.4%)	3,996 (50.1%)
Gestational age (weeks)	39.3 ± 1.8	39.4 ± 1.7
Preterm (<37 weeks)	180 (6.8%)	477 (6.0%)
Birthweight (g)	3398 ± 542	3407 ± 531
BW/GA z-score[a]	0.06 ± 0.95	0.04 ± 0.96
Mother's age	28.9 ± 5.9	29.2 ± 5.7
Mother's pre-pregnancy BMI	25.0 ± 5.6	24.3 ± 5.1
Mother's pre-pregnancy weight status		
Underweight	132 (4.9%)	369 (4.6%)
Normal weight	1,504 (56.2%)	5,004 (62.8%)
Overweight	616 (23.0%)	1,587 (19.9%)
Obese	425 (15.9%)	1,010 (12.7%)
Maternal weight gain during pregnancy (lbs)	32.4 ± 16.0	32.9 ± 15.0
Maternal weight gain during pregnancy[b]		
Less than recommended	551 (20.6%)	1,552 (19.5%)
Recommended	744 (27.8%)	2,317 (29.1%)
More than recommended	1,382 (51.6%)	4,101 (51.4%)
Mother smoked during pregnancy	252 (9.4%)	500 (6.3%)
Mother's race/ethnicity		
White	1,913 (71.4%)	5,500 (69.0%)
Black	267 (10.0%)	697 (8.8%)
Asian	131 (4.9%)	536 (6.7%)
Nat Am/PacIsl/Other/Multi	230 (8.6%)	734 (9.2%)
Hispanic	136 (5.1%)	503 (6.3%)
Highest parental education level		
Less than high school	125 (4.7%)	391 (4.9%)
High school degree	525 (19.6%)	1,372 (17.2%)
Some college	584 (21.8%)	1,500 (18.8%)
College plus	1,443 (53.9%)	4,707 (59.1%)

[a] Birthweight-for-gestational age z-score; defined according to the growth curves developed by Oken.[14]

[b] According to IOM 2009 Guidelines.[17]

Table 4.3 Characteristics of exposure to antibiotics during gestation for control subjects in the Birth Defects Study (BDS) control population (N=10,647 subjects)

Exposure	n (%)
Infant exposed to antibiotics during gestation	
Not exposed	7,970 (74.9%)
Exposed	2,677 (25.1%)
Single exposure	2,044 (76.4%)
Multiple exposures	633 (23.6%)
TIMING [a, b]	
Trimester 1 Exposure	
Yes	1,074 (49.2%)
Trimester 2 Exposure	
Yes	669 (30.7%)
Trimester 3 Exposure	
Yes	685 (31.4%)
No. of trimesters exposed	
None	7,970 (78.5%)
One	1,951 (19.2%)
Two	213 (2.1%)
Three	17 (0.2%)
DURATION[c]	
Median (min, max)[d]	8 (1, 280)
1–6 days	734 (28.0%)
7–10 days	1130 (43.1%)
11+ days	757 (28.9%)
CLASS[e]	
Antibiotic NOS	1,670 (47.9%)
Penicillins	895 (25.6%)
Macrolides	535 (15.3%)
Cephalosporins	91 (2.6%)
Miscellaneous Antibacterial	105 (3.0%)
Other	194 (5.6%)
INDICATION[f]	
Respiratory infections	1,499 (43.0%)
Genitourinary infections	1,310 (37.5%)
Other	681 (19.5%)

*Core sample of 10,647.

[a] There were 496 subjects in our final sample of 10,647 where antibiotic use with uncertain exposure periods was reported. Those reports are not classified under any trimester.

[b] Percents will add to over 100% because women reported use in multiple trimesters.

[c] Duration was summed across the entire pregnancy. 56 subjects reported antibiotic use during pregnancy, but had missing values for duration.

[d] There were 8 subjects who reported antibiotic use for duration longer than 365 days. These subjects were on continuous antibiotics. Because this analysis was restricting to during pregnancy, we capped the max at 280 days (40 weeks x 7 days=280 days).

[e] N may add to more than the number of subjects because women could use antibiotics multiple times during pregnancy and the same class of antibiotic may or may not have been prescribed each time

[f] N may add to more than the number of subjects who used antibiotics because women could have multiple indications for use throughout pregnancy

Table 4.4 The association between exposure to antibiotics during gestation and SGA* for Infants in the Birth Defects Study (BDS) control population

	Model 1 OR (95% CI)	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Exposure to antibiotics during gestation	0.90 (0.77, 1.06)	0.87 (0.74, 1.03)	0.86 (0.73, 1.01)
Mother smoked during pregnancy		2.15 (1.74, 2.66)	1.58 (1.26, 1.97)
Highest parental education level			
HS degree vs. less than high school			0.85 (0.64, 1.11)
Some college vs. less than high school			0.56 (0.42, 0.75)
College plus vs. less than high school			0.40 (0.31, 0.52)

*SGA defined as <10th percentile for gestational age and gender according to the growth curves developed by Oken, et al.[14]
Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is adjusted for maternal smoking during pregnancy and parental education.

Table 4.5 The association between exposure to antibiotics during gestation and SGA stratified by smoking during pregnancy in the Birth Defects Study (BDS) control population

	Number in exposure category	Number of SGA infants	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Smokers	752	115		
Not exposed (ref)	500	80	1.00	1.00
Exposure to antibiotics during gestation	252	35	0.85 (0.55, 1.30)	0.81 (0.53, 1.25)
Non-smokers	9,895	776		
Not exposed (ref)	7,470	603	1.00	1.00
Exposure to antibiotics during gestation	2,425	173	0.88 (0.73, 1.04)	0.86 (0.72, 1.03)

Model 1 is unadjusted.
Model 2 is adjusted for parental education.

Table 4.6 The association between exposure to antibiotics during gestation and SGA stratified by sex in the Birth Defects Study (BDS) control population (n=5,399)

	Number in exposure category	Number of SGA infants	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Females	5,399	435			
Not exposed (ref)	3,996	328	1.00	1.00	1.00
Exposure to antibiotics during gestation	1,403	107	0.92 (0.74, 1.16)	0.89 (0.71, 1.12)	0.87 (0.69, 1.09)
Males	5,248	456			
Not exposed (ref)	3,974	355	1.00	1.00	1.00
Exposure to antibiotics during gestation	1,274	101	0.88 (0.70, 1.11)	0.86 (0.68, 1.08)	0.85 (0.67, 1.07)

Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is adjusted for maternal smoking during pregnancy and parental education.

Table 4.7 The association between exposure to antibiotics during gestation and birthweight (g) in the Birth Defects Study (BDS) control population, N=10,647

	Model 1 β **(95% CI)**	**Model 2** β **(95% CI)**	**Model 3** β **(95% CI)**	**Model 4** β **(95% CI)**	**Model 5** β **(95% CI)**
All infants[a]					
Unexposed	Ref.	Ref.	Ref.	Ref.	Ref.
Exposed to antibiotics	-9.18 (-32.55, 14.19)	2.10 (-17.54, 21.74)	5.38 (-14.02, 24.79)	10.99 (-8.34, 30.32)	12.27 (-6.99, 31.53)
Pre-term[b] **(<37 weeks)**	**Model 1** β **(95% CI)**	**Model 2** β **(95% CI)**	**Model 3** β **(95% CI)**	**Model 4** β **(95% CI)**	**Model 5** β **(95% CI)**
Unexposed	Ref.	Ref.	Ref.	Ref.	Ref.
Exposed to antibiotics	-57.89 (-179.94, 64.16)	-10.81 (-94.75, 73.12)	-5.27 (-88.94, 78.39)	-6.34 (-89.61, 76.93)	-6.46 (-89.84, 76.92)
Full term[c] **(≥ 37 weeks)**	**Model 1** β **(95% CI)**	**Model 2** β **(95% CI)**	**Model 3** β **(95% CI)**	**Model 4** β **(95% CI)**	**Model 5** β **(95% CI)**
Unexposed	Ref.	Ref.	Ref.	Ref.	Ref.
Exposed to antibiotics	2.34 (-18.74, 23.43)	3.82 (-16.27, 23.92)	6.90 (-12.94, 26.74)	12.81 (-6.96, 32.57)	14.44 (-5.23, 34.12)

[a] N=10,647
[b] n=657
[c] n=9,918
Model 1 is unadjusted.
Model 2 is adjusted for gestational age (weeks)
Model 3 is adjusted for gestational age, and sex.
Model 4 is adjusted for gestational age, sex, and maternal smoking during pregnancy.
Model 5 is adjusted for gestational age, sex, maternal smoking during pregnancy, and parental education.

Table 4.8a The association between exposure to antibiotics during gestation and birthweight-for-gestational age z-score* in the Birth Defects Study (BDS) control population (N=10,647)

	Model 1 β (95% CI)	**Model 2 β (95% CI)**	**Model 3 β (95% CI)**
Unexposed	**Ref.**	**Ref.**	**Ref.**
Exposure to antibiotics during gestation	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.07)	0.03 (-0.01, 0.07)

*birthweight-for-gestational age z-score calculated using growth curves developed by Oken, et al.[14]
Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is adjusted for maternal smoking during pregnancy and parental education.

Table 4.8b The association between exposure to antibiotics during gestation and birthweight-for-gestational age z-score* stratified by smoking status during pregnancy in the Birth Defects Study (BDS) control population

	Model 1 β (95% CI)	**Model 2 β (95% CI)**
Smoker, n=752		
Unexposed	**Ref.**	**Ref.**
Exposure to antibiotics during gestation	-0.03 (-0.17, 0.11)	-0.02 (-0.17, 0.12)
	Model 1 β (95% CI)	**Model 2 β (95% CI)**
Non-smoker, n=9,895		
Unexposed	**Ref.**	**Ref.**
Exposure to antibiotics during gestation	0.03 (-0.02, 0.07)	0.03 (-0.01, 0.07)

*birthweight-for-gestational age z-score calculated using growth curves developed by Oken, et al.[14]
Model 1 is unadjusted.
Model 2 is adjusted for highest parental education.

Table 4.9a Timing of exposure to antibiotics during gestation and the association with SGA in the BDS control population (N=10,151)*

Timing[a]	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Trimester 1				
Not Exposed	9,077	1.00	1.00	1.00
Exposed	1,074	0.95 (0.76, 1.20)	0.93 (0.74, 1.17)	0.93 (0.74, 1.17)
Trimester 2				
Not Exposed	9,482	1.00	1.00	1.00
Exposed	669	1.06 (0.81, 1.40)	1.04 (0.79, 1.37)	1.01 (0.76, 1.33)
Trimester 3				
Not Exposed	9,466	1.00	1.00	1.00
Exposed	685	0.84 (0.63, 1.14)	0.83 (0.61, 1.11)	0.82 (0.61, 1.11)

*Subjects with uncertain exposure periods were removed from this analysis.
[a]The OR for exposure in each trimester is adjusted for exposure in the other two trimesters.
Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is adjusted for maternal smoking during pregnancy and parental education.

Table 4.9b Timing of exposure to antibiotics during gestation and the association with SGA in the BDS control population (n=9,921) (restricting to infants exposed to antibiotics in only one trimester at any time during gestation)

Timing[a]	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Trimester 1				
Not Exposed	9,018	1.00	1.00	1.00
Exposed	903	0.94 (0.73, 1.21)	0.92 (0.71, 1.18)	0.92 (0.71, 1.19)
Trimester 2				
Not Exposed	9,405	1.00	1.00	1.00
Exposed	516	1.04 (0.76, 1.43)	1.01 (0.74, 1.39)	0.98 (0.71, 1.34)
Trimester 3				
Not Exposed	9,389	1.00	1.00	1.00
Exposed	532	0.75 (0.53, 1.07)	0.74 (0.52, 1.05)	0.73 (0.51, 1.04)

*Subjects with uncertain exposure periods were removed from this analysis.

[a]The OR for exposure in each trimester is adjusted for exposure in the other two trimesters.

Model 1 is unadjusted.

Model 2 is adjusted for maternal smoking during pregnancy.

Model 3 is adjusted for maternal smoking during pregnancy, and parental education.

Table 4.10 Duration of exposure to antibiotics during gestation and the association with SGA in the BDS control population

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95%CI)
Duration				
0 days	7,970	1.00	1.00	1.00
1–6 days	734	0.71 (0.52, 0.97)	0.70 (0.51, 0.95)	0.70 (0.52, 0.96)
7–10 days	1,130	0.95 (0.75, 1.19)	0.92 (0.73, 1.16)	0.90 (0.71, 1.13)
11+ days	757	1.00 (0.77, 1.31)	0.95 (0.73, 1.25)	0.93 (0.71, 1.21)

Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is adjusted for maternal during pregnancy and parental education.

Table 4.11a Class of antibiotic taken during gestation and the association with SGA among infants exposed to antibiotics during gestation in the BDS control population (N=10,647)

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Type (class)[a]				
Penicillins				
No antibiotic use (ref.)	7,970	1.00	1.00	1.00
Penicillin	789	0.97 (0.75, 1.27)	0.94 (0.72, 1.23)	0.94 (0.72, 1.23)
Non-penicillin	1,888	0.87 (0.72, 1.05)	0.84 (0.70, 1.02)	0.82 (0.68, 0.99)
Macrolides				
No antibiotic use (ref.)	7,970	1.00	1.00	1.00
Macrolides	420	0.59 (0.38, 0.91)	0.59 (0.38, 0.91)	0.65 (0.42, 1.01)
Non-Macrolides	2,257	0.96 (0.81, 1.14)	0.92 (0.78, 1.10)	0.89 (0.75, 1.06)
Cephalosporins				
No antibiotic use (ref.)	7,970	1.00	1.00	1.00
Cephalosporins	84	0.40 (0.12, 1.26)	0.41 (0.13, 1.29)	0.45 (0.14, 1.42)
Non-Cephalosporin	2,593	0.92 (0.78, 1.08)	0.89 (0.75, 1.04)	0.87 (0.74, 1.02)
Antibiotic-NOS				
No antibiotic use (ref.)	7,970	1.00	1.00	1.00
Antibiotic-NOS	1,420	0.97 (0.79, 1.19)	0.93 (0.75, 1.14)	0.87 (0.71, 1.07)
Antibiotic recalled	1,257	0.82 (0.66, 1.03)	0.81 (0.64, 1.02)	0.84 (0.66, 1.05)
Miscellaneous antibacterials				
No antibiotic use (ref.)	7,970	1.00	1.00	1.00
Misc. antibacterial	97	0.70 (0.31, 1.61)	0.70 (0.31, 1.61)	0.69 (0.30, 1.60)
Non-misc. antibacterial	2,580	0.91 (0.77, 1.07)	0.88 (0.74, 1.03)	0.86 (0.73, 1.02)

[a]Analyzed as five separate models.
Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is adjusted for maternal smoking during pregnancy, and parental education.

Table 4.11b Type of antibiotic taken during gestation and the association with SGA among infants exposed to antibiotics during gestation in the BDS control population

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Type (class)				
No antibiotic use	7,970	1.00	1.00	1.00
Penicillins	789	1.00 (0.76, 1.32)	0.97 (0.74, 1.28)	0.97 (0.74, 1.28)
Non-penicillins	1,888	0.92 (0.58, 1.46)	0.90 (0.57, 1.42)	0.89 (0.74, 1.41)
Macrolides	420	0.64 (0.36, 1.12)	0.65 (0.37, 1.14)	0.73 (0.42, 1.29)
Cephalosporins	84	0.43 (0.13, 1.41)	0.46 (0.14, 1.49)	0.50 (0.15, 1.64)
Antibiotic NOS	1,420	1.07 (0.67, 1.68)	1.05 (0.66, 1.66)	0.99 (0.63, 1.57)
Misc. antibacterials	97	0.79 (0.33, 1.90)	0.81 (0.34, 1.95)	0.80 (0.33, 1.94)

Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is adjusted for maternal smoking during pregnancy, and parental education

*parameters for macrolides non-users, cephalosporin non-users, antibiotic-NOS non-users, miscellaneous antibacterial non-users get dropped out of model because they are a linear combination of other parameters (i.e., macrolides non-users are penicillin users+ penicillin non-users – macrolide users).

Table 4.12a Reason for antibiotic taken during gestation and the association with SGA among infants exposed to antibiotics during gestation in the BDS control population

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Type (class)				
Other (ref.)	1,434	1.00	1.00	1.00
Respiratory Infections	1,243	0.72 (0.54, 0.96)	0.73 (0.55, 0.98)	0.80 (0.60, 1.08)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Type (class)				
Other (ref.)	1,568	1.00	1.00	1.00
Genitourinary Infections	1,109	1.40 (1.05, 1.86)	1.36 (1.02, 1.81)	1.18 (0.88, 1.59)

Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is maternal smoking during pregnancy and parental education.

Table 4.12b Reason for antibiotic taken during gestation and the association with SGA among infants in the BDS control population

	n	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Type (class)				
Respiratory Infections				
Unexposed to antibiotics	7,970	1.00	1.00	1.00
Respiratory Infections	1,243	0.74 (0.59, 0.94)	0.73 (0.57, 0.93)	0.76 (0.60, 0.97)
Other infection	1,434	1.04 (0.85, 1.26)	1.00 (0.82, 1.22)	0.93 (0.76, 1.14)
	n	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
Type (class)				
Genitourinary Infections				
Unexposed to antibiotics	7,970	1.00	1.00	1.00
Genitourinary infections	1,109	1.08 (0.87, 1.34)	1.03 (0.83, 1.28)	0.93 (0.74, 1.16)
Other Infections	1,568	0.77 (0.63, 0.96)	0.76 (0.61, 0.94)	0.80 (0.64, 0.99)

Model 1 is unadjusted.
Model 2 is adjusted for maternal smoking during pregnancy.
Model 3 is maternal smoking during pregnancy and parental education.

Table 4.13a First trimester antibiotic use: Exploring antibiotic class, indication for use, and duration of use in the BDS control population (For subjects with multiple uses in a single trimester, only the first use is considered) (n=9,035)

	n	No. SGA	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Class				
*Non-users**	7,970	683	1.00	1.00
Penicillins	277	27	0.92 (0.56, 1.50)	0.91 (0.56, 1.50)
Macrolides	127	4	0.21 (0.07, 0.64)	0.23 (0.08, 0.69)
Antibiotic NOS	523	45	0.75 (0.44, 1.28)	0.74 (0.43, 1.26)
Other	138	10	0.51 (0.21, 1.21)	0.53 (0.22, 1.28)
Duration				
*0 days (non-users)**	7,970	683	1.00	1.00
1–6 days	298	27	1.65 (0.97, 2.81)	1.62 (0.95, 2.77)
7–10 days[a]	573	40	-----	-----
11+ days	194	19	1.66 (0.89, 3.08)	1.75 (0.94, 3.26)
Indication for use				
*Antibiotic non-users**	7,970	683	1.00	1.00
Genitourinary infection	453	38	1.06 (0.61, 1.82)	0.94 (0.54, 1.64)
Respiratory Infection[b]	408	29	-----	-----
Other	204	19	1.24 (0.64, 2.40)	1.31 (0.68, 2.54)

*Antibiotic non-users are subjects who did not report use in any trimester.

[a, b]These covariates were dropped from the model because they were linear combinations of other covariates.

Model 1 is unadjusted.

Model 2 is adjusted for smoking and parental education.

Table 4.13b Second trimester antibiotic use: Exploring antibiotic class, indication for use, and duration of use in the BDS control population (For subjects with multiple uses in a single trimester, only the first use is considered) (n=8,639)

	n	No. SGA	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Class				
*Non-users**	7,970	683	1.00	1.00
Penicillins	189	15	0.61 (0.31, 1.21)	0.63 (0.32, 1.25)
Macrolides	96	8	0.56 (0.23, 1.38)	0.65 (0.27, 1.61)
Antibiotic NOS	314	32	0.56 (0.28, 1.11)	0.57 (0.29, 1.15)
Other	70	4	0.25 (0.07, 0.88)	0.28 (0.08, 1.00)
Duration				
*0 days (non-users)**	7,970	683	1.00	1.00
1–6 days	222	24	1.73 (0.92, 3.24)	1.72 (0.91, 3.22)
7–10 days[a]	349	25	-----	-----
11+ days	98	10	1.65 (0.74, 3.66)	1.49 (0.66, 3.33)
Indication for use				
*Antibiotic non-users**	7,970	683	1.00	1.00
Genitourinary infection	242	31	2.41 (1.22, 4.75)	1.94 (0.97, 3.87)
Respiratory Infection[b]	291	19	-----	-----
Other	136	9	1.00 (0.43, 2.33)	0.93 (0.40, 2.20)

*Antibiotic non-users are subjects who did not report use in any trimester.

[a, b]These covariates were dropped from the model because they were linear combinations of other covariates.

Model 1 is unadjusted.

Model 2 is adjusted for smoking and parental education.

Table 4.13c Third trimester antibiotic use: Exploring antibiotic class, indication for use, and duration of use in the BDS control population (For subjects with multiple uses in a single trimester, only the first use is considered) (n=8,655)

	n	No. SGA	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Class				
*Non-users**	7,970	683	1.00	1.00
Penicillins	196	20	1.03 (0.57, 1.89)	1.06 (0.58, 1.95)
Macrolides	97	7	1.03 (0.42, 2.54)	1.12 (0.45, 2.79)
Antibiotic NOS	330	20	0.48 (0.23, 1.01)	0.47 (0.22, 0.99)
Other	62	3	0.42 (0.11, 1.61)	0.50 (0.13, 1.91)
Duration				
*0 days (non-users)**	7,970	683	1.00	1.00
1–6 days	295	19	0.64 (0.33, 1.24)	0.70 (0.36, 1.36)
7–10 days[a]	320	29	-----	-----
11+ days	70	2	0.26 (0.06, 1.15)	0.25 (0.06, 1.12)
Indication for use				
*Antibiotic non-users**	7,970	683	1.00	1.00
Genitourinary infection	234	22	2.71 (1.28, 5.71)	2.26 (1.07, 4.76)
Respiratory Infection[b]	300	18	-----	-----
Other	151	10	1.56 (0.66, 3.69)	1.37 (0.58, 3.24)

*Antibiotic non-users are subjects who did not report use in any trimester.

[a,b]These covariates were dropped from the model because they were linear combinations of other covariates.

Model 1 is unadjusted.

Model 2 is adjusted for smoking and parental education.

4.5 APPENDIX

Appendix 1.

Accutane
Acetaminophen
Actron
Acyclovir
Advair
Advil
Aerobid
Afrin
Albuterol
Aleve
Alka-seltzer
Allegra
Amitriptyline
Aspirin
Azmacort
Bayer
Beclomethasone
Bufferin
Celexa
Chlor-trimeton
Claritin
Cromolyn
Cymbalta
Datril
Dristan
Ecotrin
Effexor
Elamar
Elavil
Excedrin
Fever relievers
Foradil
Ibuprofen
Intal
Lexapro
Luvox
Motrin
Naprosyn
Nasalcrom
Nuprin
Orudis
Other acetaminophen products
Other aspirin products
Other ibuprofen products
Other Naproxen products
Other pain products
Panadol
Paxil
Pepto Bismol
Peramivir
Pristiq
Prozac
Relenza
Retin-A
Serevent
Singulair
Slo-bid
Sudafed/pseudophedrine
Symbicort
Tamiflu
Theo-dur
Tylenol
Tylenol products
Valcyclovir
Valtrex
Wellbutrin
Zoloft
Zovirax
Zyban
Zyrtec

Appendix 2.

V1:
- Ampicillin
- Amoxicillin
- Augmentin
- Erythromycin
- Bactrim
- Septra
- Sulfamethoxazole
- Flagyl
- Protostat
- Metronidazole
- Zithromax

V11:
- Ampicillin
- Amoxicillin
- Augmentin
- Erythromycin
- Bactrim
- Flagyl
- Metronidazole
- Zithromax

V20.6:
- Amoxicillin, Ampicillin, Penicillin (Trimox), Dicloxicillin
- Zithromax, Z-Pak
- Augmentin (amoxicillin-clavulanate)
- Bactrim (TMP/trimethoprim/sulfamethoxazole)
- Erythromycin (EC, EES)
- Macrobid (Nitrofurantoin)
- Keflex (Cephalexin)
- Ceclor (Cefaclor)
- Clindamycin (Clindamycin)
- Biaxin (Clarithromycin)
- Cipro (Ciprofloxacin)
- Flagyl (Metronidazole)

V21:

Amoxicillin, Ampicillin, Penicillin (Trimox), Dicloxicillin
Zithromax, Z-Pak
Augmentin (amoxicillin-clavulanate)
Bactrim (TMP/trimethoprim/sulfamethoxazole)
Erythromycin (EC, EES)
Macrobid (Nitrofurantoin)
Keflex (Cephalexin)
Ceclor (Cefaclor)
Clindamycin (Clindamycin)
Biaxin (Clarithromycin)
Cipro (Ciprofloxacin)
Flagyl (Metronidazole)

Topical Antib.:
Benzamycin, Akne-mycin, T-stat
Cleocin T
Benzaclin
Metrogel (Metronidazole)

Appendix 3. List of Antibiotic Classes

Amebicides
Aminoglycosides
Anti-bacterials
Anti-mycobacterials
Anti-protozoals
Anti-tuberculosis
Cephalosporins
Macrolides
Antibiotic-NOS (not otherwise specificed)
Miscellaneous anti-mycobacterials
Penicillins
Quinolones
Sulfonamides
Tetracyclines

Appendix 4. List of Indications for Antibiotic Use

Respiratory:
Cold, flu, cough, bronchitis, pneumonia, sinus infection, congestion
The flu
Cold, cough, bronchitis, pneumonia, sinus infection, congestion
Allergies
*allergy with cold
Ear infection
*ear infection with cold
Strep throat
*strep throat with cold
Asthma
*asthma with allergy
*asthma with cold
*asthma, allergy, cold

Genitourinary:
urinary tract infection
vaginal infection
genital herpes
other STD
genital herpes (recurrence)
gynecological procedure
other menstrual problems

Other:
Chicken Pox
Antibiotics/med for infection
Toxemia/Preeclampsia/HELLP syndrome
Oral herpes/cold sores
Nausea and/or vomiting
Vaginal bleeding during pregnancy
Stomach flu/virus
Fever at any time
Nausea
Diarrhea
Medicine for hospital prior to delivery
Infertility
Other medical procedures
Infertility procedure
Other indication
toothache
Joint or muscle pain/sprains/injury
Other pains
Itching
Ulcers
Other stomach problems
To prevent miscarriage
To stop labor or contractions
Acne
Other skin problems

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5. CONCLUSION

The studies in this dissertation explored three different exposures at three distinct periods in childhood and the corresponding associations with weight or biomarkers of metabolic status. We examined the association between PA and MetS among overweight adolescents ages 12–19, the association between ETS and overweight among young children ages 3–6, and then association between exposure to antibiotics in utero and small for gestational age.

In our first study examining the association between PA and MetS among 12–19-year-old adolescents in the NHANES study population, we observed that modest amounts of MVPA were associated with a reduced risk of MetS. The inverse relationship was present among subgroups of boys, girls, and those with and without food insecurity. We observed that vigorous physical activity was driving this association. Our study population was racially and ethnically diverse, and one of the first studies we are aware of to consider the effects of sexual maturation in females on the association between PA and MetS. We observed that whether the female had experienced early menarche (<12) did not appreciably alter the association between PA and MetS. The cross-sectional design of our study was a limitation; however, we believe reverse causality is an unlikely explanation for our observed results given the similar findings reported by prospective observational cohort studies[1–3] and the similar inverse association observed in each population subgroup when we stratified on obesity severity.

In our second study examining the association between ETS and overweight among 3–6 year olds in NHANES, we found a positive association between ETS and risk

of overweight/obesity, with a dose-response relationship observed. Given the cross-sectional nature of our design, we cannot establish temporality of this association; however, we believe reverse causality is unlikely given that our results were consistent with findings observed by other prospective observational cohort studies.[4–6] A major strength of our study was our ability to use serum cotinine to define the exposure rather than relying on self-report of tobacco use by adults in the household. Since this exposure is often underreported, exposure measurement error is a real concern of prior studies. Serum cotinine also captures average daily exposure and doesn't limit the exposure assessment to the child's primary residence. In our study, we examined the association between self-reported smokers in the household and serum cotinine levels. While the two were highly correlated, we observed that a significant percentage of subjects with the highest serum cotinine levels were from households that reported no smokers, meaning that children were being exposed to ETS outside of the home. The dose-response relationship observed between serum cotinine and child overweight/obesity was not detected using the self-reported exposure assessment, validating concerns about misclassification of tobacco use and underreporting of use in prior studies. Although we could not rule out the influence of residual and unmeasured confounding, the biologic rationale exists to suggest that the inflammatory effects of tobacco smoke influence the child's weight and biomarkers of metabolic health status.[4, 6–7]

Lastly, in our third study, we observed that infants exposed to antibiotic use in utero had a reduced risk of SGA. We did not observe an association with birthweight or BW/GA-z score as has been reported in other studies.[8–11] Our study is the largest we are

aware of that has explored this relationship. Previous studies on maternal antibiotic use during pregnancy and birthweight have used different outcomes (SGA, low birthweight, birthweight adjusted for gestational age, and BW/GA-z score) making cross comparisons difficult.[8–11] Additionally, these studies have been limited in the characteristics of antibiotic exposure that they can assess. With our data, we were able to evaluate trimester of exposure, duration, class, and indication for use. We observed that the association between class, duration, and indication for use with SGA varied substantially across trimesters.

In summary, we explored exposures at different stages of childhood and development and their relationship with weight and metabolic biomarkers. We were able to study PA and MetS in a racially and ethnically diverse population and study the effects of sexual maturation in females on this association, addressing limitations of previous studies. We also studied the association between ETS and overweight using a biologic marker of ETS exposure rather than relying on self-report, reducing measurement error that has plagued prior studies. Lastly, our study of maternal antibiotic use during pregnancy and SGA was one of the largest studies we are aware of that explored this relationship. The results of these studies serve as a complement to the growing body of literature on pediatric obesity.

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